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SOYBEAN SEED QUALITY AS AFFECTED BY TIME OF PLANTING IN THE DRY ZONE OF SRI LANKA*

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Abstract : Eight soybean (*Glycine max* (L.) Merr.) cultivars were planted during the first week of each calender month in the dry zone of Sri Lanka. The soybean crops established from May and June plantings produced yield and size of seed significantly greater than the other plantings. These seeds, which matured under more favourable weather conditions with the combination of daily mean temperature (28°F) and daily maximum relative humidity (75%), were of the highest quality as indicated by their high level of viability and vigour. In contrast, the crop of January and February plantings gave seeds of lowest quality and at the time of their maturation the daily mean temperature was remarkably high (30 – 31°C). The predicted model explaining the effect of weather factors on seed quality reveals that daily mean temperature and daily maximum relative humidity are the significant attributes to seed quality of soybean.

1. Introduction

Weather is probably the preharvest factor that has the greatest influence on seed quality in many crops, including cereals and legumes. Marked weather injuries in wheat seeds have been attributed to results from temperature[12] or preharvest rains.[11] Exposure to dampness[6] or hot weather[9] during maturation could lead to the production of low quality seeds in soybean.

There are distinct advantages in producing soybean seeds under favourable weather conditions and such conditions are rather uncommon in the subtropics and tropics.[1] Under favourable conditions, seed set and recovery is usually optimum; there is low incidence and severity of pests and diseases and germination and seed vigour are quite high. Hence, seed quality is usually good if seeds are produced under favourable conditions. In soybean growing areas of Sri Lanka, the prevailing practice has been to plant soybean in the early part of May and November which are the beginning of dry and wet seasons, respectively. However, it could be possible to extend the

* Part of Ph.D. thesis submitted by senior author, to the Postgraduate Institute of Agriculture of the University of Peradeniya, Sri Lanka.

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planting time within these growing seasons. This is important for various reasons which include unavailability of seed material and labour at the peak time of seasons and crop failure due to uncertain weather. Therefore, an investigation was undertaken to determine the most appropriate time of planting for quality seed production in soybean in the dry zone of Sri Lanka. This investigation also attempts to critically analyse the major weather factors that are responsible for seed quality of soybean.

2. Experimental

The experiment consisted of eight soybean cultivars (Bossier, Improved Pelican, M 534, Pb–1, PM 78–13, PM 78–25, UPSL 216, 30120–49–3) arranged in a randomized complete block design with four replications. The block was 27.5m x 1.6m in size and consisted of eight plots. Each plot had four 3 m rows spaced at 40 cm, having plants at 5 cm apart. The seeds of these cultivars were planted on the fifth day of each month for 12 months (May 1984 to April 1985) at the Agricultural Research Station, Maha Illuppallama, which is situated in the dry zone of Sri Lanka. Maha Illuppallama, being at a latitude of $8^{o}5$'N, could be classified as a humid tropical area. The experiment was well managed by providing the optimum conditions for growth and development. Seeds were harvested from two centre rows of each plot after 3 – 3½ months, at development stage R_8 at which 95% of the pods were mature.[8] Harvested seeds were sun-dried to a seed moisture content of 10% and stored under cold storage at 10°C and 45% relative humidity until assayed for viability and vigour. The seed yield and the weight of 100 seeds were also recorded.

From seed lots produced by each planting date, 25 seeds from each cultivar and each replicate were drawn at random for testing of seed quality. These seeds were mixed with Captan 80% WP (N[(Trichloromethyl) thio]–4–cyclohexane 1, 2 dicarboximide) at 3 g/1 kg of seeds and planted in moist sterilized sand contained in plastic boxes. Seeds were germinated under illumination at 8 h a day, using flourescent lamps of 750–1000 lux placed 1 m above the sand boxes, and at a constant temperature of 30 ± 1°C, the known approximate optimum for soybean seed germination.[5] Another 25 Captan-treated seeds of each sample were planted in the field where conditions were favourable for germination.

Seedlings that emerged were counted five and eight days after planting in both germination tests. Seedlings were carefully uprooted eight days after planting, examined and classified in accordance with the criteria of the Association of Official Seed Analysts.[2] Seedling length and dry weight were determined using five seedlings taken at random from each sample. Seedling length was measured from the point of attachment of the cotyledons to the

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root tip. The same seedlings were used to obtain the dry weight gravimetrically after drying in an oven at 60°C for 48 h.

Percentage germination was used as a measure of seed viability. Reported germination values include only normal seedlings counted after eight days. The seedling length and dry weight were the parameters assigned to estimate seed vigour. In addition, the emergence rate was computed as a modification of the method suggested by Maguire[10] using the formula given below:

$$\frac{1}{2}\left[\frac{\text{number of seedlings emerged on 5th day after planting}}{5} + \frac{\text{number of seedlings emerged on 8th day after planting}}{8}\right]$$

The data obtained on viability and vigour of seed lots harvested at different times were processed and subjected to the standard analysis of variance. Germination percentages were transformed to arcsine degree scale before statistical analysis. Means were compared using Duncan's Multiple Range Test.[7] Correlations were carried out to see whether seed yield could be considered as a guide to predict seed quality. Regressions were computed as appropriate.

3. Results and Discussion

Results indicate a considerable variation in yield, size and quality of seeds produced from soybean crops that were established from different planting dates. The mean germination of seed lots of eight cultivars in sand was over 91.8% for the plantings of May, June and July. These values were significantly higher than % germination of seed lots from other plantings, except for the August and November plantings (Table 1). Likewise, the field germination of seeds produced from the plantings of May, June and July was over 89.8% and significantly higher than the other plantings, with the exception of August planting (Table 1). The % germination of January and February plantings was lowest in sand germination test[9] while field germination test showed lowest germination in February and March plantings. These results showed that seeds produced from field plantings made during the period May to July were generally of high viability.

Emergence rates higher than 3.0 per day were noticed in the plantings made from May to August and also in November in both sand and field germination and these values did not differ significantly from each other (Table 1). Lowest values for emergence rate were obtained for the plantings of January, February and March.

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Table 1. Mean germination percentages, emergence rate, seedling length and dry weight of eight soybean cultivars from 12 different plantings in sand (SG) and field (FG) germination tests.

Planting time		% germination		Emergence rate (day^{-1})		Seedling length (cm)		Seedling dry weight (mg)	
		SG	FG	SG	FG	SG	FG	SG	FG
1984	May 5	96.7a	94.8a	3.7a	3.6a	26.4a	11.6abc	97.5a	137.9ab
	June 5	92.3ab	89.8ab	3.6a	3.6a	21.4bcd	12.4a	99.3a	148.6a
	July 5	91.8ab	90.3ab	3.7a	3.6a	22.6abc	12.5a	87.9b	118.8bcd
	August 5	87.4b	83.6bc	3.7a	3.3a	26.4a	12.1ab	82.5b	114.1cde
	September 5	75.2c	55.1de	3.2bc	2.2bc	19.0cd	11.6abc	88.1b	126.9bc
	October 5	68.6cd	57.5d	2.9cd	2.5bc	22.4abc	11.8ab	84.8b	88.2fgh
	November 5	86.4b	76.4c	3.7a	3.1a	17.3de	10.6bc	100.8a	101.1def
	December 5	47.6e	49.6e	2.4e	2.0c	19.0cd	9.9cd	69.2c	84.6ghi
1985	January 5	24.9f	30.7f	1.5f	1.2d	7.2f	6.6f	41.8d	61.5hi
	February 5	30.8f	6.6h	1.9f	0.2f	9.8f	7.3ef	28.9e	84.3ghi
	March 5	59.6d	12.6gh	2.7de	0.5ef	17.1de	7.4ef	51.3d	94.8efg
	April 5	57.6de	17.8fg	2.6de	0.8de	18.8cd	8.4de	66.4c	110.8cde

Mean followed by the same letters in each column do not differ significantly, based on Duncan's Multiple Range Test at P = 0.05

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The highest mean seedling length was recorded for May and August plantings in sand germination. In field germination, maximum length was observed from July planting; however, it did not differ statistically from May and June plantings(Table 1). The highest mean seedling dry weight was for the November planting in sand germination which however, did not show any significant difference from plantings made in May and June (Table 1). With respect to seedling dry weight under field conditions, May and June plantings showed the highest weight. The above data generally suggest that the seeds produced from plantings made during the period May to July and also in November have good seed vigour.

Table 2. Mean seed yield and size of eight soybean cultivars harvested from 12 different plantings in one year.

Planting time		Mean seed yield	Mean seed size
Year	Date	(Kg/ha)	(g/100 seeds)
1984	May 5	4842.5 a	15.4 a
	June 5	4765.1 a	15.2 a
	July 5	3028.9 b	12.6 b
	August 5	2255.4 d	12.6 b
	September 5	843.4 h	11.1 bc
	October 5	1301.9 f	11.8 bc
	November 5	2882.6 c	13.3 b
	December 5	1120.0 g	11.1 c
1985	January 5	684.6 i	11.6 bc
	February 5	486.6 j	6.4 d
	March 5	1547.7 e	11.8 bc
	April 5	2249.4 d	12.9 b

Mean followed by same letters in each column do not differ significantly at P = 0.05 based on Duncan's Multiple Range Test.

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The highest seed yield was also obtained from May and June plantings which had the highest seed size as well (Table 2). Seed yield and seed size of these plantings were found to be significantly greater than those of other plantings, including November planting which is the regular planting time during the wet season. Considering the collective data on seed yield, seed size, seed viability and vigour together, the best time of planting for seed production appears to be the period during May to June in the dry zone of Sri Lanka. High quality seeds that were produced from May and June plantings matured under more favourable weather conditions with the combination of daily mean temperature of about 28°C and daily maximum relative humidity of 75–78% (Table 3). In contrast, seeds of plantings in January and February which were of lowest quality (Table 1), matured when the daily mean temperature was highest (30–31°C). Several workers have pointed out earlier that adverse weather conditions during seed maturation could cause severe seed quality problems which reduce seed viability and vigour in soybean.[3,4,6,11] Gregg[9] reported that dry hot weather and inadequate soil moisture during seed maturation resulted in green seeds of soybean which were of low quality with respect to germability.

Table 3. Mean temperature and maximum relative humidity during seed maturation of the soybean cultivars planted at 12 different planting times of the year.

Planting time Year	Date	Mean temperature (°C)	Maximum relative humidity (%)
1984	May 5	28.2	78.4
	June 5	28.4	74.9
	July 5	26.8	89.6
	August 5	27.2	82.6
	September 5	24.8	90.9
	October 5	25.9	92.7
	November 5	25.9	91.7
	December 5	27.2	88.0
1985	January 5	30.0	80.1
	February 5	31.0	75.5
	March 5	28.3	82.1
	April 5	28.4	83.6

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Seed yield was significantly and positively correlated with germination, emergence rate, seedling length and dry weight (Table 4). The close association of seed yield with seed quality characteristics such as seed viability and vigour suggests that seed yield may be considered as a useful guide to predict seed quality.

Table 4. Correlation between seed yield and parameters of viability and vigour of seeds of different plantings.

Correlated characters	Correlation coefficient
Seed yield (486.6 – 4842.5 Kg/ha)	
Vs	
Germination (23.3 – 97.4%)	0.7649++
Emergence rate ($1.46 - 3.70\ day^{-1}$)	0.6223++
Seedling length (7.2 – 26.4 cm)	0.6904++
Seedling dry weight (28.9 – 100.8 mg)	0.7263++

++ Significant at P = 0.01

A multiple linear regression model was used to estimate the effect of weather parameters that prevailed during the period from physiological to harvest maturity on seed quality (Y) in terms of seed viability (% germination in sand at 30°C and field) and vigour (emergence rate) (Table 5). The weather parameters included in the estimation were daily maximum relative humidity (XI), daily minimum relative humidity (X2), daily mean relative humidity (X3), daily mean temperature (X4), daily rainfall (X5) and daily sunshine hours (X6). From the available data, the predicted model by estimation is given as:

$$Y = B_o - B_1X_1 - B_2X_2 + B_3X_3 - B_4X_4 + B_5X_5 + B_6X_6.$$

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Table 5. Estimates of the coefficients of regressions for variables in the model for determining the effect of weather parameters, during the period from physiological to harvest maturity (seed maturation), on seed quality.

Variable	% germination in sand at 30°C	% germination in field	Emergence rate in sand at 30°C
Intercept	299.9035	61.3811	10.1062
Daily maximum RH (%) (X1)	− 1.4968++ (0.4366)	−1.0305+ (0.5726)	−0.0442++ (0.0134)
Daily minimum RH (%) (X2)	− 0.1473 (0.3370)	−0.8422 (0.4944)	−0.0036 (0.0116)
Daily mean RH (%) (X3)	0.9721 (0.6878)	1.0892 (0.9020)	0.0307 (0.0212)
Daily mean temperature (8°C) (X4)	− 6.1626+++ (0.9993)	−7.1747+++ (1.3105)	−0.2016+++ (0.0307)
Daily rainfall (mm) (X5)	0.2513 (0.1626)	0.2568 (0.1739)	0.0078 (0.0061)
Daily sunshine hours (X6)	0.3303 (0.6181)	0.6972 (0.8113)	0.0151 (0.0191)
Coefficient of correlation (r)	0.4434	0.3724	0.4742

+, ++, +++ Significant at P = 0.05, 0.01, 0.001, respectively.

Standard error of estimates are given in parenthesis under coefficient of regression values.

The model indicates that daily mean temperature and daily maximum relative humidity had a significant influence on seed viability and vigour. The other parameters considered did not influence seed quality significantly (Table 5). The regression coefficient values predict that % germination in sand and field respectively decrease by 6.1626 and 7.1747 for each °C increase in daily mean temperature and 1.4968 and 1.0305 for each % increase in daily maximum relative humidity (Table 5). Emergence rate which measures seed vigour declines by 0.0442 and 0.2016 per day for each

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% increase in daily maximum relative humidity and each ^{o}C increase in daily mean temperature (Table 5). The data obtained reveal that daily mean temperature and daily maximum relative humidity during the period from physiological to harvest maturity of soybean crop are the most important determinants of seed quality.

4. Conclusions

Collective data on yield, size, viability and vigour of seeds produced from different plantings suggest that the best time of planting for the production of maximum yield of high quality soybean seed in the dry zone of Sri Lanka would be during the period of May to June when moderate daily mean temperature (28°C) and daily maximum relative humidity (75%) prevail during seed maturation. In contrast, seeds from January and February plantings which mature under high daily mean temperature (30–31°C) would be of lower quality. The data also show that, of the weather factors, daily mean temperature and daily maximum relative humidity during seed maturation are the probable major determinants of soybean seed quality.

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SOME OBSERVATIONS ON THE DOWNY MILDEW DISEASE OF GRAPE VINE CAUSED BY *PLASMOPARA VITICOLA* IN JAFFNA

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Abstract : Frequent field observations made in the vineyards of Jaffna showed that the disease is confined to the rainy season of the year. The development of symptoms on various parts of grape vine has been observed. Invasion of the host tissue was accompanied by changes in colour and appearance of the affected portion. Sporangia of *Plasmopara viticola* germinated after 18 h of incubation at $25^{o} \pm 1^{o}C$ and 100% r h and their single thick germ tubes penetrated the stomata by about 48 h. Penetration strictly occurred through stomata. Following penetration the fungus formed intercellular hyphae and colonised the spongy and pallisade parenchyma cells of the host. The external appearance of sporulating structures took about five days and this occurred strictly through the stomata. The lesions remained productive upto the ninth day during incubation.

1. Introduction

Grape vine *(Vitis vinifera)* cultivation in Sri Lanka has been a success and the grapes produced in the vineyards of Jaffna and the eastern regions are sufficient to meet the needs of the whole country. In tropical climates the grape vine is evergreen and it has been found in the Jaffna region that by forcing the vine into two growth cycles, one in the wet season and the other in the dry season, it produces profitably.

The most destructive fungal disease of grape vine is the downy mildew caused by *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni. This disease has been observed in almost all the vineyards in the Jaffna region during the rainy season and periods of dew from October to March. The development of the fungus was favoured by the high humidity and low temperature of rainy weather and the presence of dew. There was a gradual weakening of the plants due to infection and the yield was reduced because of the severe yearly attacks by the fungus. There have been instances where grape cultivation was completely abandoned as a result.

The present investigation was made in order to obtain basic information with regard to the occurrence and biology of the fungus in the vineyards in the Jaffna region. This is indeed the first report of such a study in Sri Lanka on the downy mildew disease of grape vine.

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2. Experimental

Frequent field observations were made in the vineyards of Jaffna and information regarding the occurrence of the disease and the development of symptoms was recorded.

Following the observations on the development of the symptoms on various parts of the grape vine, the different developmental stages of the fungus, namely the germination of sporangia, internal colonization and sporulation, were examined in detail under laboratory conditions.

For the laboratory experiments healthy mature leaves were collected from the field, brought to the laboratory and maintained at a temperature of $25^{o}C \pm 1$ in an illuminated incubator (Gallenkamp Cooled Incubator . . . . .). These materials were used as the source of host tissue throughout the experiment. All observations were made on the grape vine variety Israel blue which is the commonly cultivated variety in Sri Lanka.

Inoculum was obtained from mature vine leaves infected with *Plasmopara viticola*. These affected leaves collected from the field were placed in pairs, under surface to under surface, in plastic boxes and kept in refrigerators at $4^{o}C$. When required these leaves were taken out and shaken in a petri dish of sterile distilled water (SDW).The sporangial suspension thus prepared was used to inoculate fresh sets of leaves or leaf discs of 2 cm diameter. The concentration of the sporangial suspension was adjusted to $2.4 x 10^{7}$ sporangia ml^{-1} with SDW.

Inoculation was done by spraying the sporangial suspension with a hand sprayer. Leaf discs of 2 cm diameter or entire leaves were inoculated on the lower surface and kept on moist filter paper discs in Petri dishes. The Petri dishes were incubated at $25^{o} \pm 1^{o}C$ and at 100% rh in an illuminated incubator.

Assessment of the development of the fungus was one as follows:-

2.1 Germination of sporangium

Five inoculated leaf discs were removed from the illuminated incubator at two-hour intervals during incubation and sellotape impressions of the inoculated surface were prepared. The preparations were stained with cotton blue in lactophenol and mounted in 50% glycerine on a clean glass slide. The impressions were observed under a light microscope (x 400) for germination of sporangia. The numbers of germinated and ungerminated sporangia were determined from counts of 200–300 sporangia in five microscopic fields, randomly selected from each of the five leaf discs. A sporangium was

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considered to have germinated when the germ tube length exceed its breadth.[4] From the above data the mean percentage germination of sporangia was determined. The length of germ tube of about fifty germinated sporangia was measured after 24 hours of incubation, under the microscope (x 400) with the use of a calibrated eye-piece graticule.

2.2 Infection

Observations were made on hand sections and scrapings of the host leaf, taken at six-hour intervals after incubation. The time of appearance of initial symptoms and formation of visible lesions were noted.

The amount of infection was determined at 24-hour intervals after inoculation by measuring the diameter of visible lesions. The lesions were assumed to be circular and the diameter of each lesion was measured thrice at right angles to each other, under the microscope (x 40). The amount of infection was expressed as mean percentage area of infection taken from sets of five leaves.

2.3 Sporulation

The inoculated leaf discs were incubated at $25^{o} \pm 1^{o}C$ and 100% r h sets of five leaf discs were removed from the illuminated incubator at six-hour intervals commencing 48 hours after inoculation and assessed for sporulation, by the following two methods:

2.3.1 Sporangiophore production

The incubated leaf discs were boiled in 1:1 acetic acid–acetone mixture in a water bath for 15 minutes to decolourise the host tissue. After decolourisation the host material was placed on a glass slide, stained with cotton blue in lactophenol, mounted in glycerine and covered with a clean cover slip. These preparations were observed under the microscope and the number of sporangiophores present per microscopic field (x 40) was determined. These values were then converted to number of sporangiophores produced per mm^2 area of the host tissue.

2.3.2 Sporangia production

The incubated leaf discs were shaken with one ml of SDW in Mc Carty bottles for one minute. The numbet of sporangia present in the sporangial suspension was determined by using a haemocytometer. These values were then converted to number of sporangia produced per cm^2 area of the leaf surface.

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2.4 Perennation of the fungus

The perennation of the fungus during unfavourable seasons has in most cases been ascribed to the production of oospores as reported by Lafon & Built.[3] Attempts were made to look for these by periodical sectioning of infected leaves fallen on the ground and leaf debris during the disappearance of the fungus.

3. Results

3.1 Development of symptoms on grape vine

All green parts of the grape vine, namely the leaves, tendrils, stems, inflorescences and berries were found to be affected by the fungus. Invasion of the host tissue was accompanied by changes in colour and appearance of the affected portion. The colour changes and symptoms produced on various parts of grape vine are given below:

3.1.1 Leaves

The leaf was found to be the most susceptible tissue to fungal attack. The first evidence of infection was the appearance of light yellow translucent spots or 'oil spots' on the upper leaf surface resulting from the internal colonization by the fungus within the host tissue. The leaves later became mottled and soon white patches of downy growth of the sporulating structures were formed on the lower surface of the leaves. These were due to the sporangiophores and sporangia of the fungus. The sporangiophores were branched and were of determinate growth. These were found to arise singly or in tufts from the epidermis strictly through the stomata. The branches of sporangiophores were found to arise perpendicularly from the main axis; the branchlets produced single elliptical sporangia at their terminal ends, on sterigmata. The mature sporangiophores were hyaline and did not take up the stain cotton blue in lactophenol.

When the disease became severe in a vineyard, the lower surface of the leaves was fully covered with the fungus. The lesions covered with the sporulating structures turned brown and finally became necrotic. Badly infected leaves became dry and crumpled and finally dropped from the plant. Vine plants that had been poorly cared for were completely defoliated due to the infection.

3.1.2 Stems

Stems were found to be affected only during growth up to a distance of about 80–100 mm from the apex. The infected portions of the stems deve-

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loped brown streaks and at later stages of infection they became hooked at the tips. The nodes were found to be more susceptible than the internodes. No sporangiophore production was observed on the surface of the stems.

3.1.3 Tendrils

Young tendrils were infected by the fungus and showed symptoms of infection as brown streaks. The symptoms first appeared at the tips and then gradually spread to other portions of tendrils. Here too the tendrils became hooked at the tips at later stages of infection. No external production of sporangia was observed.

3.1.4 Inflorescence

Brown irregular patches or longitudinal streaks appeared on the inflorescence axes due to fungal attack; very often the infected inflorescence curled up, became dry and failed to develop fruits. On the inflorescence axis no external production of sporangia was observed. The affected inflorescences were often shed from the plant.

3.1.5 Berries

Downy mildew attack on the berries was apparent from the initial formation until ripening. After flowering, the developing bunches which were subjected to fungal attack showed yellowish brown patches of infection due to the penetration of the fungus through stomata of the stalks and the fruits. As the berries matured, the diseased parts became brown but at no stage was external production of sporangia observed. However, white masses of sporulating structures were produced on the stalks of the berries where the stomata present were still functioning. Berries severely affected at an early stage failed to develop further, but when affected at a later stage of development became shrivelled and were found to be shed easily from the plants. During the period of investigation it was not possible to observe the white cottony growth of the mildew on parts other than leaves in the fields.

3.2 Germination of Sporangium

Sporangial suspensions when examined under the microscope showed presence of three stages of sporangia depending on the maturity. Sporangia of the first category were immature, small (20–26 μm in length and 12 – 16 μm in breadth), finely granular and were filled with inclusions. These sporangia readily took up the stain – cotton blue in lactophenol. Their hyaline wall was thin and uniform in thickness. Papillae were not distinct in these sporangia. These sporangia were capable of germinating after about 48 hours of incubation. Sporangia of the second category were fairly

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mature (24 – 32 μm in length and 15 – 18 μm in breadth), slightly brownish with dense inclusions and oil globules and were found to germinate directly within 24 hours forming single thick germ tubes. Sporangia of this type and their germ tubes readily took up the stain. The germ tubes were found to arise from the papillary ends. Sporangia of the third category were large (30 – 37 μm in length and 15 – 18 μm in breadth) brownish and coarsely granular. These did not germinate directly by producing single germ tubes; instead, they germinated by producing zoospores. Presence of zoospores was only observed in the sporangia of this category. Counts on the number of zoospores present in these sporangia indicated that about 4 – 16 zoospores were usually produced from a single sporangium, irrespective of the conditions in which these sporangia were formed. The zoospores were about 2 μm in diameter on an average. The sporangia have been observed to burst open at the papillary end and the zoospores were found to escape through this opening. The liberated zoospores have been observed to reach and germinate on the lower leaf surface of vine leaves. Germination by zoospore formation has been found to be less frequent than direct germination of sporangia. In all experiments on germination, the germination of only the second category of sporangia was assessed.

In the study of germination of sporangia, inoculated leaf discs incubated at 25° ± 1°C and at 100% r h in an illuminated incubator were assessed on the germination after short periods Sporangia started to germinate after about 16 hours producing thick, single germ tubes. However, a considerable number of sporangia was found to have germinated 18 hours after incubation. The germination values increased significantly up to 48 hours (Table 1).

The length of germ tube of germinated sporangia increased with increase in period of incubation (Table 1) but since the germinated sporangia were found to reach and penetrate through the stomata or perish after 48 hours, it was difficult to make further measurement on the germ tube length after 48 hours of incubation.

3.3 Internal colonization

The germ tubes that penetrated the stomata were found to reach the substomatal cavity on the lower surface of the host leaf. Observations showed that on the lower leaf surface, one stoma was penetrated by about three germ tubes on an average but the number varied from 1 to 5. The average number of stomata per microscopic field (x 400) on the lower surface of the host was found to be 6 and about 50% of the stoma were penetrated. It was also observed that the penetration of the host occurs strictly through stomata. Direct penetration through the epidermis or by other means has not been observed.

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Table 1. The germination of sporangia and growth of germ tube of *Plasmopara viticola* on detached leaves of grape vine at 25°C and 100% relative humidity.

Period of incubation (hours)	Mean % germination	Mean length of germ tube (μ m).
10	0	0
12	0	0
14	0	0
16	3.3 a	25
28	39.5 b	42
20	42.2 c	83
22	44.6 d	91
24	45.8 e	103
48	51.6 f	164
72	51.4 f	166

Note :— Values denoted by different letters are statistically different.

The germinated sporangia which entered the host tissue formed intercellular hyphae and colonised the spongy and the palisade parenchyma cells of the host. Globular haustoria with short necks were observed inside these host cells.

Following the internal colonization, the host tissue changed in colour from green to pale yellow depending on the severity of infection. This discolouration or the appearance of oil spots of infections on the upper leaf surface was observed 60 hours after incubation and was the first visible symptom of infection.

The mean percentage area of infection increased with increase in period of incubation and reached 100% by the twelfth day after incubation (Table 2).

3.4 Sporulation

Following internal colonization, the emergence of sporangiophores took place through the stomata on the lower leaf surface. The first emergence of sporangiophore initials was observed around 65 hours after incubation, and clusters of sporangiophores continued to emerge out of the stomata after 72 hours of incubation. About 25% of the total stomata present in a micros-

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Table 2. Development of infection by *Plasmopara viticola* on detached leaves of grape vine at 25°C and 100% relative humidity.

Period of incubation (days)	Mean % area of infection
0	0
1	0
2	0
3	0
4	2.1
5	28.6
6	52.0
7	86.4
8	98.1
9	100.0
10	100.0

copic field (x 400) showed emergence of sporangiophore initials. Mature sporangiophores were present only on the fifth day after incubation. The mature sporangiophores were branched or unbranched and varied in axial length from 116 μm – 286 μm and in breadth from 16 – 26 μm. They produced 3 – 7 branches and 3 – 7 branchlets on each branch. The mature sporangiophores emerged out of the stomata at the rate of about 4 per stoma on an average but the number varied from 1 – 12 per stoma, on a lesion that was five days old.

In such a lesion, about 90% of the total stomata in a microscope field (x 400) showed presence of sporangiophores. The number of immature sporangiophores increased up to the sixth day after incubation and gradually decreased thereafter. At the same time the number of mature sporangiophores increased up to the eighth day and then became static (Table 3). By this time the lesion had attained its full growth.

The length measurements of sporangiophores showed that the sporangiophores increased in length with time from the third day after incubation. The increase was rapid up to the eighth day and thereafter the length of the sporangiophore remained more or less constant (Table 4).

Sporulation or sporangia production was measured by determining the amount of sporangia of the three categories produced per sq. mm area of the lesion (Table 5). The amount of immature sporangia produced was high on the fifth day after incubation and thereafter it started decreasing. From the

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Table 3. The production of sporangiophores of *Plasmopara viticola* on detached leaves of grape vine at 25°C and 100% relative humidity.

Period of incubation	Mean number of sporangiophores/ mm^2 area of host leaf		
	i.s.	m.s.	Total
3	53	0	53
4	107	10	117
5	140	80	220
6	180	210	390
7	145	364	514
8	10	570	580
9	0	570	570

i.s. = Immature sporangiophores.
m.s. = Mature sporangiophores.

Table 4. Growth of sporangiophore of *Plasmopara viticola* on detached leaves of grape vine at 25°C and 100% relative humidity.

Period of incubation (days)	Length of sporangiophores (m).		
	i.s.	m.s.	Mean
3	56	0	56
4	122	147	124
5	123	270	176
6	122	277	205
7	138	282	260
8	0	286	286
9	0	285	285
10	0	283	283
11	0	285	285

i.s. = Immature sporangiophores.
m.s. = Mature sporangiophores.

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Table 5. Production of sporangia of *Plasmopara viticola* on detached leaves of grape vine at 25°C and 100% of relative humidity.

Period of incubation (days)	Mean number of sporangia produced/ mm^2 area of host leaf.			
	i.	f.m.	m.	Total
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	93	0	0	93
5	912	480	0	1392
6	860	2100	363	3323
7	582	2400	2600	5582
8	286	2750	4980	8016
9	105	3890	4873	8768
10	0	2600	2722	5322
11	0	2100	2093	4193

i. = Immature sporangia
f.m. = Fairly mature sporangia
m. = Mature sporangia.

fourth day onwards the number of fairly mature sporangia increased in number and reached a maximum value after nine days The amount of fairly mature sporangia then decreased slowly and became static on the eleventh day. Similarly the amount of mature sporangia present increased from the fifth day and reached its maximum value between the eighth and the ninth days. The value became static on the eleventh day after decreasing slowly.

During these investigations oospores have never been observed though attempts were made to look for them.

4. Discussion

It has been observed for several years that the downy mildew disease of grape vine is a destructive fungal disease. This is usually severe during periods of high humidity and low temperature or rainy weather conditions.

The fungus responsible for grape vine downy mildew *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni. is an obligate parasite which cannot

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therefore be easily cultivated *in vitro* or on inert nutritive media.

Observations on the occurrence of downy mildew in the vineyards showed that infections begin in September or October when the rainy season starts and last till April with the greatest severity of the disease observed in late February or March.

Almost all green parts of the vine were affected by this fungus as already observed by Ramanathan and Sivapalan,[6] but the most prominent symptoms were on leaves. It was suggested by Rives and Lafon[8] that only the young actively growing organs are susceptible to infection. The infections occurred on the lower leaf surface and undoubtedly through the stomata, as already confirmed by Ravaz and Verge.[7]

The first symptom of infection was the formation of translucent yellow 'oil spots' on the upper surface which is followed by appearance of a white downy growth of sporulating structures on the under leaf surface corresponding to the oil spots. Apart from leaves, the berries, tendrils, inflorescences and stems too developed infections[5] but at no stage was external production of sporangiophores observed on parts other than leaves during this study. Infection on these parts appeared as only brown, irregular or linear patches or streaks as a result of internal colonization. However there are reports by several authors on the formation of sporangiophores and sporangia on the surface of berries and inflorescences.[3] But these workers claim that these organs are susceptible as long as the stomata on them remain functioning for the reason that penetration of host and emergence of sporangiophores occur strictly through stomata.

Weakening of leaves due to infection led to their premature senescence. The yield and life span of the grape vine was highly reduced by the downy mildew attacks.

The infection process of the fungus was initiated by germination of sporangia during favourable conditions. Sporangia directly germinated by emitting the entire contents and forming thick germ tubes which penetrated stomata on the host leaf and colonised the inner tissues. The external appearance of sporulating structures through stomata of the lower leaf surface took about five days and lesions remained productive up to the ninth day.

The perennation of the fungus during unfavourable seasons has been attributed by several workers[3] to the sexual reproductive structures known as the oospores. Observations towards the end of the season failed to reveal the presence of oospores or the mode of perennation of the fungus. The disease is completely absent during the season from May to September. In these circumstances the mode of overwintering may be by preservation of mycelia which remain between the bud scales in diseased plants until bud-burst as suggested by Galet[2] and Boubals.[1]

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MID—HOLOCENE SEA LEVEL CHANGES IN SRI LANKA

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Abstract : Sea level changes due to various factors cause shifts in the coastline of Sri Lanka, as in other regions of the World. They are valuable in understanding the long-term factors that cause coastal erosion. The Mid-Holocene sea level changes were studied using geomorphological maps aided by field work and a laboratory analysis of materials. The bay beaches, mid-bay bars, barriers, spits, shell beds, wave-cut terraces, etc., which originated from an early marine transgression were observed along the inland areas of the Kalametiya, Lunama and Koholankala lagoons and the Minihagalkanda beds of the Southeast coast. They submerged the land to form deep embayments and barrier chains. Information obtained through radiocarbon dating and previous studies prove that these sea levels prevailed during 4040 ± 70 BP to 3620 ± 70 BP.

Introduction

Ever since the beginning of the Holocene period, fluctuations in sea levels have changed the positions of many coasts of the world. Rising sea levels and falling sea levels are normally referred to as marine transgressions and regressions respectively, and these events cause degradation and progradation of a coast, by submergence and emergence.

The principal factors causing sea level changes on a global scale can be summarized as follows;

a) Long-term tectonic changes causing the relative sea level to rise or fall. This is known as tectono-eustacy.

b) Alternating glaciation and deglaciation affecting changes of ocean water. During glaciation, there is an increased concentration of water on land resulting in a proportional drop in sea level. This is called glacio-eustacy.

c) Unloading and loading of ice from or on the Earth's crust leads to gravitational disequilibrium between different rock strata. This is known as glacio-isostasy. Glacio-isostasy may cause a coast to emerge or submerge. Similarly, hydro-isostasy refers to unloading and loading of water from or on ocean floors, leading to gravitational disequilibrium. This long-term process can also lead to emergence or submergence of coasts.

Seasonal changes in the mean sea level of the ocean are caused by the combined effect of factors such as tidal changes, changes in local atmospheric pressures, changes in wind velocity and direction, melting of ice or

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freezing of water anywhere, etc, and the result is the seasonal emergence and submergence of a strip of beach.

During the last five millenia, the coastal zone of Sri Lanka has been subjected to various geomorphological changes governed by sea level changes resulting submergence and emergence of land strips. Early bays in Mid-Holocene have been cut off by barrier chains and they formed into lagoons, while the barriers are being crowned by elongated continuous ridges of dunes. Changes of river outfalls and obstruction of estuaries can be observed along the Southwest, Southeast and Northeast coast of Sri Lanka. In addition, the Southwest coast is endangered by coastal erosion, losing extensive land portions,[11] and during the last two centuries, for example, some headlands, points, extensive sandy beaches, etc, have been completely washed away.[17] Several causes have contributed to this such as geological, geomorphological, anthropogenic, etc.[13] The sea level changes since the Mid-Holocene are one of the most effective factors of these changes in Sri Lanka. Therefore, a study of sea level changes of the Island since the Mid-Holocene will contribute towards understanding the causal factors on a long-term basis.

The position of the coastline of Sri Lanka fluctuated substantially during the Quaternary Era and studies have been conducted or mentioned by Wayland,[16] Coates,[2] Deraniyagala,[5] Swan,[11,12] and Cooray.[3] However, no attention has been paid to the position of the Mid-Holocene (since 5000 BP) with the aid of geomorphological evidence and radiocarbon dating of samples.

2. Area

The coastal zone stretching from Kalametiya to Minihagalkanda covers long and diversified tracts of coastal environments. The present study concentrates upon three selected coastal tracts, namely, the Kalametiya-Lunama lagoons, the Koholankala lagoon and the Minihagalkanda beds.

Lying in the southeastern extremity of Sri Lanka, the study area is dominated by marine influences operating on deeply weathered, metamorphic rocks that have been levelled to a slightly to moderately dissected planation surface that have been called 'the coastal peneplain' by Adams[1] and Wadia.[15]

The geomorphology of the area is characterized by a series of depositional coastal features in the littoral zone and the adjacent hinterland and by fluvial deposits and two recognizable planation surfaces in the hinterland. The planation surfaces formed in Precambrian and Palaeozoic metamorphic

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rocks can be observed to form headlands at several places in the littoral zone.

The coastal area is covered by predominantly Quaternary deposits that can be found along beaches, lagoons, estuaries, lakes and in river flood plains as well as the slopes of planation surfaces. A large part of the study area is covered by beaches and dune sands forming a belt of unconsolidated materials parallel to the coast. The beach sands are not restricted to the littoral zone, but can also be found in the fossilized beaches at some places in the immediate hinterland. In subrecent times, chains of barriers have formed closing the lagoons.[19] In later periods, most of these barriers were reworked by wind action. At many places, therefore, the dunes are situated on barriers.

3. Materials and Methods

Evidences derived from genetic geomorphological surveys compiled into a map of an area reveals former land forms which originated from marine and other processes. Therefore, such a survey would recognize former coastlines resulting from higher sea levels. The former coastlines associated with beaches, barriers, spits, etc, denote the sea level raised by early marine transgressions. Thus geomorphological maps consisting of former landforms and coastal evolutionary stages were studied so as to demarcate the position of the former coastline.

The method of mapping landforms of an area by the ITC*system of geomorphological survey, uses aerial photographs and other remote sensing techniques.[14,18] This system is an applied geomorphological discipline which provides a concise and systematic picture of landforms and related phenomena of an area. According to this method, aerial photographs i.e. B & W Panchromatic infrared, False colour and satellite imagery i.e. Landsat 3, SPOT or radar imagery as required, are interpreted using a mirror stereoscope and other instruments, taking into consideration the tone, mottling pattern, contrast, texture, etc, of the photographs or imagery. The interpreted features are normally elaborated by field work and/or laboratory analysis of materials. A geomorphological map so compiled shows types of landforms, morphometric, morphogenetic and morphochronological properties of landforms as well as a classification of landforms by origin into structural, denudational, marine, fluvial, aeolian, etc, under a well arranged key. Therefore, the ITC system serves to understand morphology, morphometry, genesis, as well as the chronology of the landforms of an area using geomorphological maps.

The geomorphological maps studied according to the ITC system were from Kalametiya-Lunama lagoons,[21] the Koholankala lagoon,[20] and the

*International Institute for Aerospace Survey and Earth Sciences (ITC)

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Minihagalkanda beds.[19] The landforms of the maps have been classified according to the ITC systems and field work has been carried out so as to check the landforms included on maps, especially those that originated from marine action, and also to collect representative samples. The clarification of the landforms included on maps and the correct identification of landforms were also made possible through field work.

The representative samples were studied at the sedimentological laboratory at the Department of Geography, University of Colombo, using standard laboratory procedures in grain size analysis and clay/silt content. Samples weighing 30 g were treated with H_2O_2 (30%) and HCl (10%) so as to remove organic matter and carbonates, respectively. The samples were then washed and wet-sieved using a 0.062 mm sieve, the pan fractions being funneled directly into Liter sedimentation cylinders. Next, peptizer (Na_4P_2O $IOH_2O + Na_2CO_3$) was added and the cylinders were filled to the litre mark. After shaking, 20 ml were removed from the cylinders and placed in nickel containers. After drying, the (silt+clay) contents were determined by weighing. The remaining 980 ml suspensions were shaken again and allowed to settle. After some 16 hours, 20 ml samples were again drawn from the cylinders, dried and weighed. The clay (and silt + clay) contents were then calculated using the formula:

$$\frac{\text{observed weight (g)} - 800\text{ (mg)}}{\text{absolute dry weight (g)}} \times 50 \times 100\%$$

The mg represent the weight of the peptizer per 20 ml sample. The difference in weight between (clay+silt) and clay is the silt content. The sand fraction (> 0.062 mm) was dried in an oven for 24 hours at 105°C. An electric sieve shaker with 10 sieves (1.41, 1.00, 0.707, 0.500, 0.354, 0.250, 0.177, 0.125, 0.088 and 0.062 mm) was used to separate the grains according to size. Then the fractions were weighed.

The grain size distribution of a clastic sediment is a measure of the depositing medium and the energy of the basing.[8] The depositing medium (or process) can be recognized if the grain size distributions are drawn into cumulative curves and histograms, etc, and then compared with each other. The technique is well explained by Reineck and Sing[8] and is also used by Swan,[12] for such studies in Sri Lanka. The laboratory data of 16 samples were drawn into cumulative curves and histograms aided by computer programme PLOTGRAINS.[6] Using the shape of the cumulative curves, and distributional patterns shown in the histogram, the origin of the material was investigated. For example, the shape of the cumulative curves of the barrier of Kalametiya and the planation surface at Batampara as shown in Figures 1 and 2 respectively, are different to each other in the shape of the cumulative

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curves and the distributional patterns shown in the histograms. The difference between the content of the silt/clay is also prominent because the processes of two landforms are different from each other. As the fossiliferous marine landforms identified from aerial photographs have been further ascertained by such a recognized geomorphological technique, the map of landforms possess a highly accurate and scientific basis. Therefore, the former landforms recognizable are used here to postulate the heights and positions of the Mid-Holocene sea levels.

In addition, the shells of window pane oysters from a former beach and a former barrier were dated using C^{14} dating at Groningen University, Netherlands, so as to determine the absolute age of the recognized sea levels. However, no absolute dates for marine transgressions and regressions have been obtained prior to this study. Some observations however can be found in a few studies.[9,10] The C^{14} dates obtained by the author can be compared with the ages of Mid-Holocene sea level changes relating to other regions of the world.

4. Results and Discussion

4.1 Evidence of Higher Sea Levels in the Kalametiya-Lunama lagoonal area

The area of the Kalametiya-Lunama lagoons as shown in Figure 3 (a & b) are characterized by former bay beaches (unit BB) with shell beds commonly known as the Hathagala beds. Around the Kalametiya lagoon, the beds are prominent with huge colonization of *Placenta placenta,* a window pane oyster. The oysters' shells with marine sediments can be observed around the Kalametiya lagoon. These beaches are now situated around the present lagoons at a maximum height of 5 m above sea level.

Even though, the longest and widest beaches of this type can be seen around the Kalametiya and the Lunama lagoons, the continuation of the early beaches which correspond with a higher sea level is evident by shells, coral fragments and beach sands at many places around the Maha Lewaya (lagoon), Mahasittarakala, Ambilikala, Koholankala, Boondala lagoons, etc.

The so-called Hathagala beds, reported by several authors,[3,4] to consist of similar materials and beaches. These beds have been interpreted by these authors as estuarine deposits similar to Beira Lake deposits in Colombo and inland estuarine deposits. However, they did not study the height and the allied geomorphological features of the beds. These shell beds are found some 1–2 km inland of the present beach and 5 m above the present sea level. The other places where such materials found are Lunama, Welipatanwila, Mahasittarakala, Malala, Udamalala, Maha Lewaya, Karagam Lewaya,

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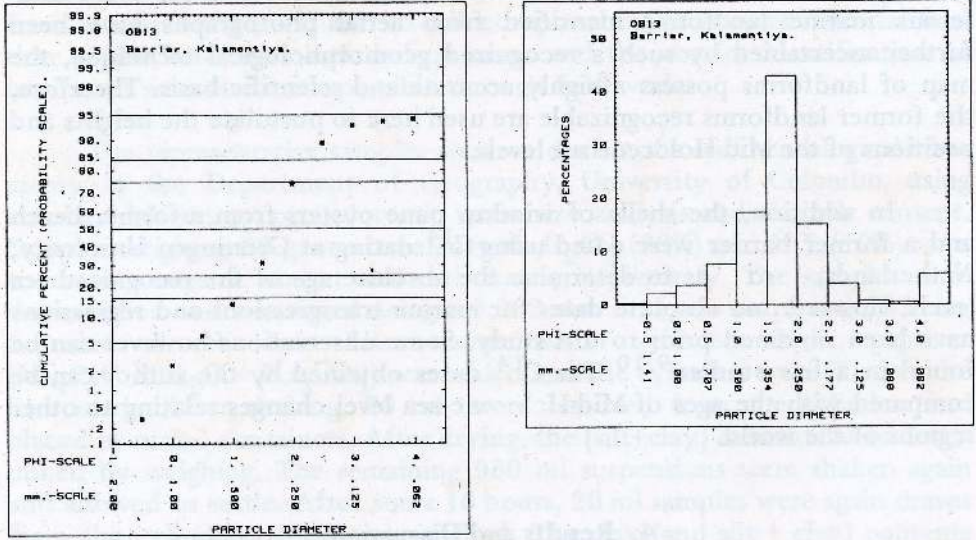

Figure 1. Grain size distribution of barrie. of Kalametiya. Silt/clay content is shown in the histogram in the shaded column.

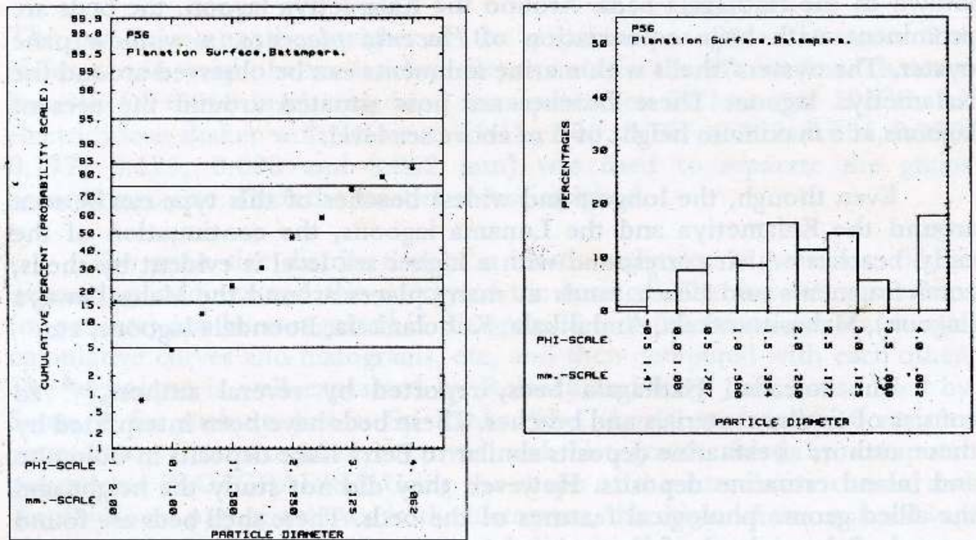

Figure 2. Grain size distribution of the planation surface at Batampara. Silt/clay content is shown in the shaded column.

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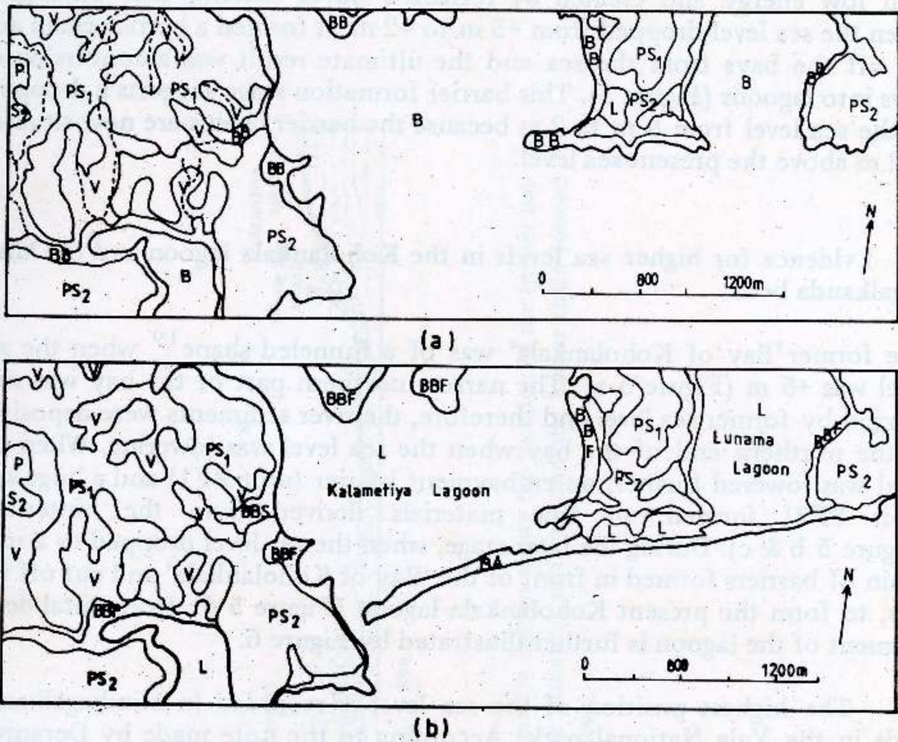

Figure 3 (a). **The Kalametiya–Lunama lagoons during the Mid-Holocene period. The sea level was 5 m above the present sea level. Edges of the planation surfaces (PS_1, PS_2) were submerged by this higher sea level forming deep embayments (B). The bays (B) were characterized by bay beaches (BB) inhabited by oyster colonies. Oyster-shells were preserved in the beds at several places, especially at Hatagala. Many streams flow from the valleys (V) into bays, and some submerged parts of the lowlands on the either side formed into small lagoons (L).**

Figure 3 (b). **The embayments were cut off by a chain of barriers (BA) when the sea level dropped from +5 m to +2m, and formed into the Kalametiya and Lunama lagoons. The present sea level is 2 m below the barrier chain.**

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Embilikala, Bundala, etc. These are evidences of a sea level at 5 m above, and the beaches were formed by early embayment environment associated with low energy and created by refracted waves entering into baylets.[19] When the sea level dropped from +5 m to +2 m, it formed a barrier chain and cut off the bays from the sea and the ultimate result was a conversion of bays into lagoons (Figure 3). This barrier formation stage suggests a dropping of the sea level from 5 m to 2 m because the barrier chains are now situated at 2 m above the present sea level.

4.2 Evidence for higher sea levels in the Koholankala lagoon and the Minihagalkanda beds

The former 'Bay of Koholankala' was of a funneled shape[19] when the sea level was +5 m (Figure 5 a). The narrow northern part of the bay was submerged by former sea level and therefore, the river sediments were deposited in the northern end of the bay when the sea level was lowering. When sea level was lowered further, an embayment barrier (unit BF1) and a huge spit (unit SF2) formed, of fine materials derived from the hinterland (Figure 5 b & c). During the later stage, when the sea level dropped to 2 m, a chain of barriers formed in front of the 'Bay of Koholankala' and cut off the bay, to form the present Koholankala lagoon (Figure 5 d). Horizontal development of the lagoon is further illustrated by Figure 6.

The highest position of the sea level is recorded in Minihagalkanda beds in the Yala National park. According to the note made by Deraniyagala,[5] the author investigated into marine terraces that may be cut off by possible early sea levels, using aerial photographs in triplets with the help of a Mirror stereoscope and a Parallex Bar, and the interpretation* is shown in Figure 7. The beds were formed during the Lower Miocene.[3] During marine transgressions three terraces were cut into the seaward edge of the beds, reflecting three main sea levels. Parallax measurements of the terraces reveal that two of them were situated more than 5 m above the present sea level. They could be Pleistocene higher sea levels marked on these Miocene beds.

The evidence derived from the Kalametiya, Lunama and Koholankala lagoons contributes to position the two sea levels at the 5 m and 2 m above present sea level. As the dating is necessary to evaluate the real age of the highest sea level, the shells collected from the Kalametiya 5 m beach, and from the mid-bay bar of the Koholankala lagoon were tested and the results can be shown as follows;

5m beach (Kalametiya bay beach)	3620 ± 70 BP #	(9–GrN–13306)
5m beach (mid-bay bar of Koholankala)	4040 ± 70 BP #	(69–GrN–13308)

*Due to insurgent activities during the field work, the author was not allowed to enter the area which is under the Department of Wildlife, therefore, field measurements could not be carried out.

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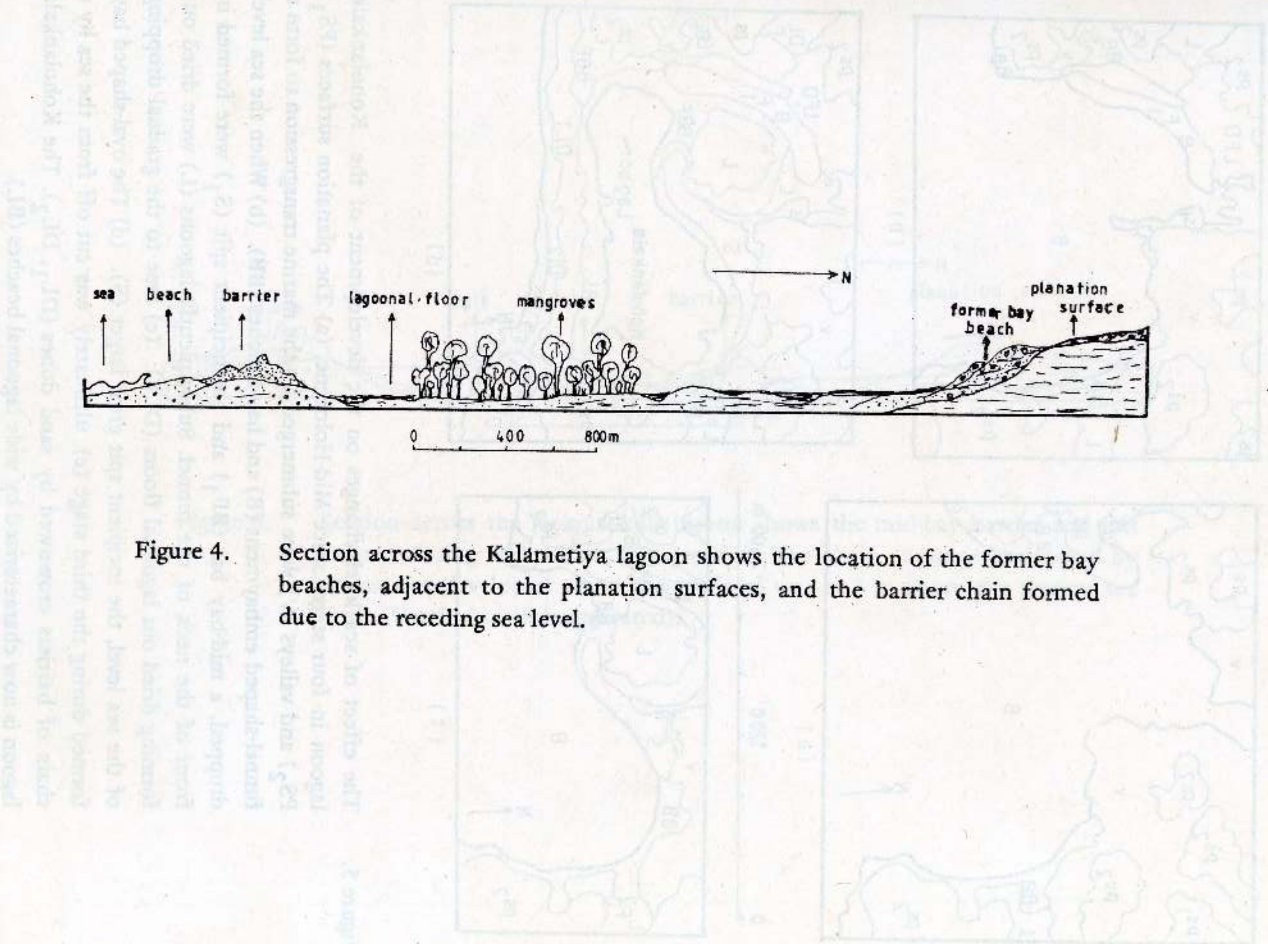

Figure 4. Section across the Kalámetiya lagoon shows the location of the former bay beaches, adjacent to the planation surfaces, and the barrier chain formed due to the receding sea level.

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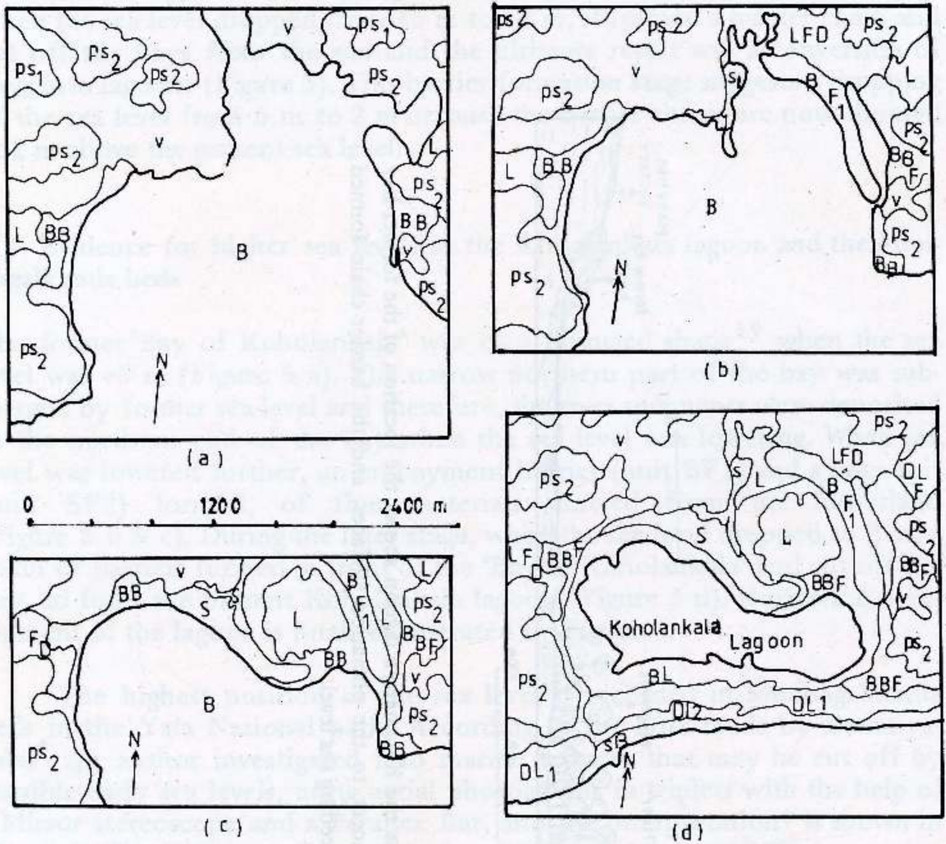

Figure 5. The effect of sea level changes on the development of the Koholankala lagoon in four stages since Mid-Holocene. (a) The planation surfaces (PS_1, PS_2) and valleys (V) were submerged by the marine transgression to form a funnel-shaped embayment (B) and bay beaches (BB). (b) When the sea level dropped, a mid-bay bar (BF_1) and an incipient spit (S_1) were formed in front of the neck of the funnel. Subsequently lagoons (L) were dried out forming dried out lagoonal floors (DLF). (c) Due to the gradual dropping of the sea level, the incipient spit grew larger (S). (d) The oval-shaped bay formed during the third stage (c), ultimately was cut off from the sea by a chain of barriers crownwed by sand dunes (DL_1, DL_2). The Koholankala lagoon is now characterized by wide lagoonal beaches (BL).

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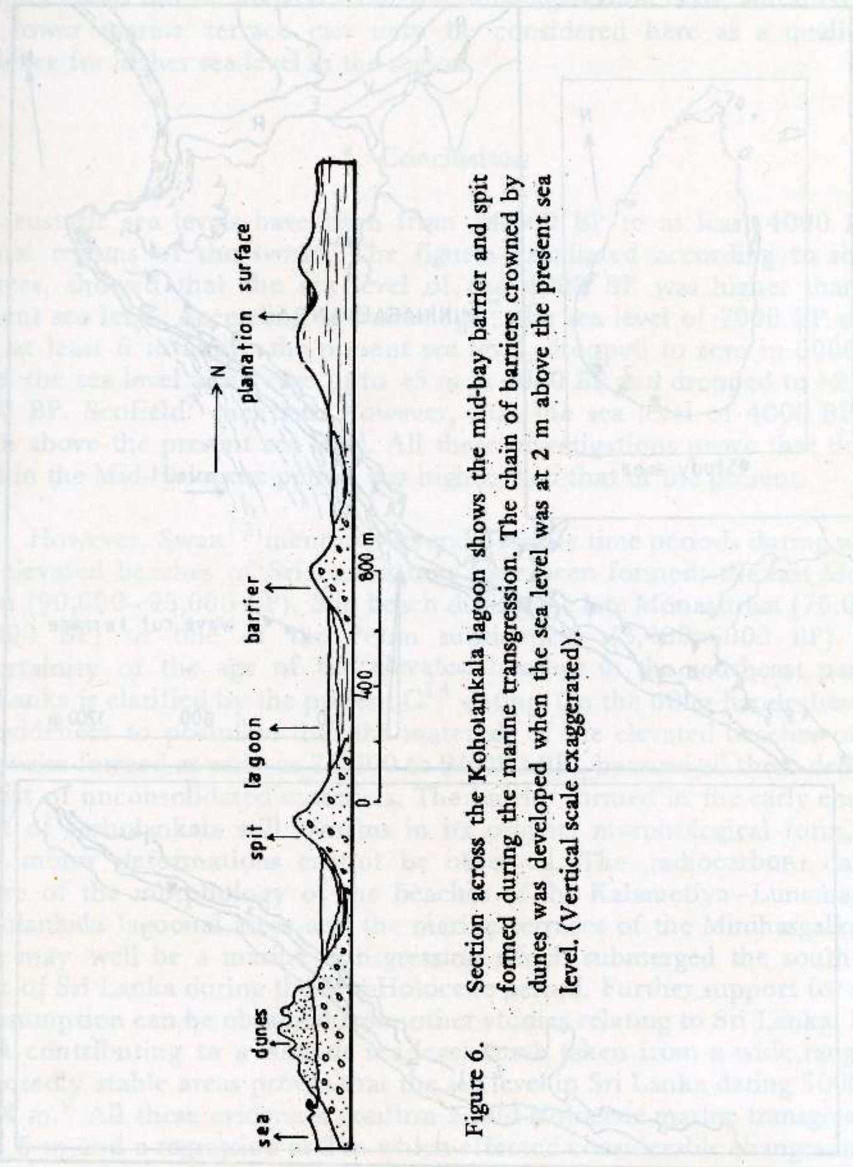

Figure 6. Section across the Koholankala lagoon shows the mid-bay barrier and spit formed during the marine transgression. The chain of barriers crowned by dunes was developed when the sea level was at 2 m above the present sea level. (Vertical scale exaggerated).

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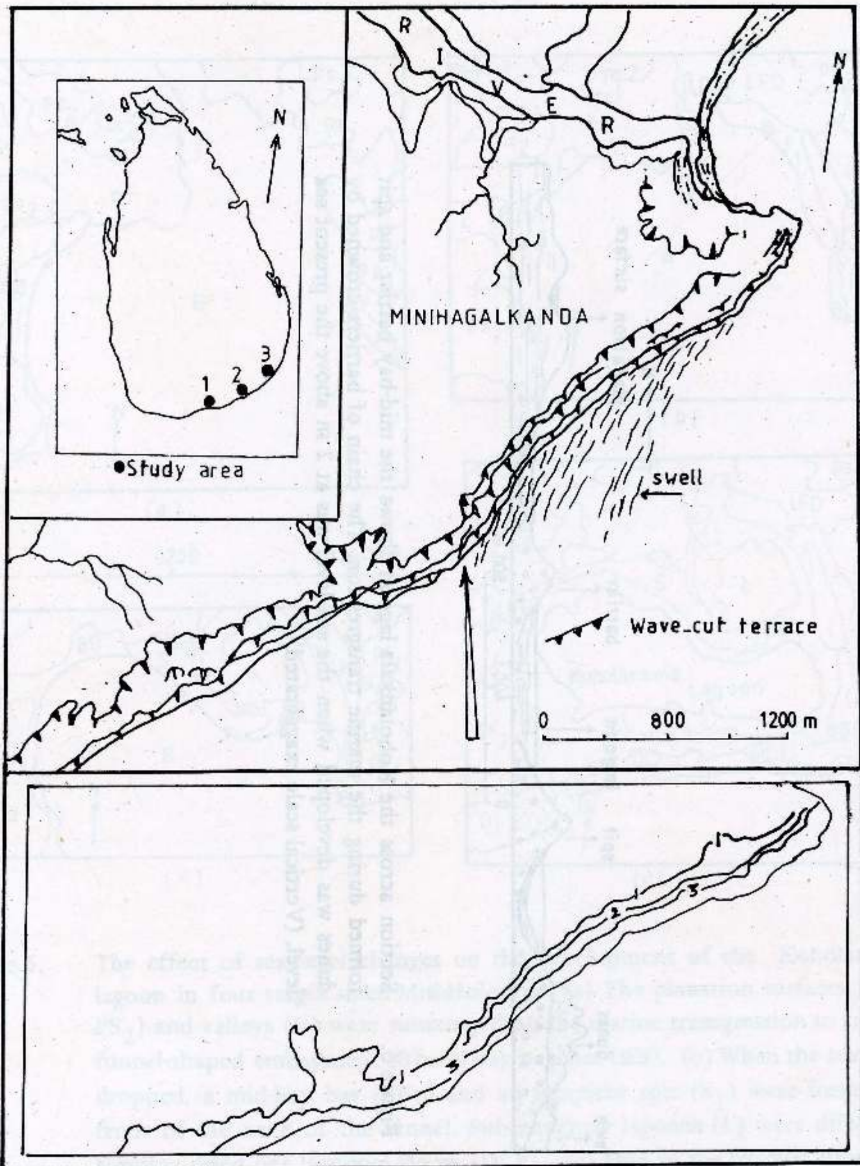

Figure 7. **The marine terraces on the Minihagalkanda beds cut by unknown early higher sea levels. The lower insert map denotes the relative elevation as 1. (high), 2. (middle), and 3 (low). The upper insert shows the location of study areas, as 1. The Kalametiya Lunama lagoons, 2. The Koholankala lagoon and 3. The Minihagalkanda beds.**

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Accordingly, the highest sea level can be dated as around 4000 BP. However, samples could not be derived from the Minihagalkanda beds, and therefore, the lower marine terrace can only be considered here as a qualitative evidence for higher sea level in the region.

5. Conclusions

The eustatic sea levels have risen from 14,000 BP to at least 4000 BP in several regions of the world. The figures calculated according to several sources, showed that the sea level of the 4000 BP was higher than the present sea level. According to Fairbridge,[7] the sea level of 7000 BP which was at least 6 m above the present sea level, dropped to zero in 6000 BP. Then the sea level again rose upto +3 m in 5000 BP and dropped to +2 m in 4000 BP. Scofield[7] mentions however, that the sea level of 4000 BP was +5 m above the present sea level. All these investigations prove that the sea level in the Mid-Holocene period was higher than that of the present.

However, Swan[12] mentions several possible time periods during which the elevated beaches of Sri Lanka may have been formed: the last Monastirian (90,000–95,000 BP), 3 m beach during the late Monastirian (75,000–80,000 BP) or one of the Peron subsidences (3,400=6000 BP). The uncertainity of the age of the elevated beaches in the southeast part of Sri Lanka is clarified by the present C^{14} dating. On the other hand, there are no evidences to postulate that the materials of the elevated beaches of the area were formed as early as 75,000 to 95,000 BP., because all these deposits consist of unconsolidated materials. The barrier formed in the early embayment of Koholankala still remains in its original morphological form, and even minor deformations cannot be observed. The radiocarbon dating, nature of the morphology of the beaches of the Kalametiya–Lunama and Koholankala lagoonal areas and the marine terraces of the Minihasgalkanda beds may well be a marine transgression which submerged the south-east coast of Sri Lanka during the Mid-Holocene period. Further support for such an assumption can be obtained from other studies relating to Sri Lanka. Data point contributing to a eustatic sea level curve taken from a wide range of supposedly stable areas proves that the sea level in Sri Lanka dating 3000 BP was 4 m.[9] All these evidences confirm a Mid-Holocene marine transgression from 5 m and a regression at 2 m which effected considerable changes in the coastline of Sri Lanka.

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EFFECT OF MATURITY ON SOME CHEMICAL CONSTITUENTS OF TURMERIC *(CURCUMA LONGA L.)*

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Abstract : The effect of maturity on rhizome yield, essential oil content and composition, and curcumin I, II and III of the main Sri Lankan cultivar of *Curcuma longa L.* is reported. The optimum time of harvest for maximum rhizome yield, was found to be 9 months. The oil content in bulbs was higher than that of finger rhizomes. The optimum time for harvest for maximum yield of turmeric oil was found to be 7.5 – 8.0 months. Essential oil composition varied with maturity, sesquiterpenes (ar-turmerone and turmerone) increased while monoterpenes (1,8 cineole and α–phellandrene) declined in both bulbs and finger rhizomes. Monoterpene content was lower in the mother sets during the early stages of growth. Curcumin (curcumin, demethoxy-curcumin, bis-demethoxycurcumin also termed curcumin I, II and III respectively) content was monitored using t.l.c-uv spectrophotometry and a technique based on t.l.c.-uv densitometry which was developed during this study. Curcumin I content was highest, however curcumin III content was more than curcumin II. Maturity did not affect the ratio of curcumins to any great extent. Advanced maturity resulted in a decline in total curcumin content. Maximum curcumin per bush was attained about 9 months after planting.

1. Introduction

Curcuma longa L. (Zingiberaceae) known as Turmeric, is cultivated widely throughout the tropics. The rhizome of this plant is conspicuous by its yellow pigment comprising the curcumins. It is an important food adjuvant and is used in indigenous medicine.[1]

Although turmeric rhizome is used the world over as a spice and food colour, the effect of maturity on essential oil content and ratio of curcumins has not been reported in any detail in the literature. According to Krishnamurthi *et al.*[5] the maximum colour varies with maturity and falls to nearly half its value if harvest is delayed.

The objectives of this study were to determine the effect of maturity on:– (i) curcumin content, (ii) the ratio of curcumins, (iii) volatile oil content, and (iv) volatile oil composition, in order to determine the time of optimum harvest considering volatile oil and curcumin yield.

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2. Experimental

2.1 Plant material

Rhizomes of the major local turmeric cultivar were planted in an experimental plot at Ceylon Institute of Scientific and Industrial Research in two trials in June 1983 and June 1984. Seven beds were prepared (3m x 1.3m). Dried cowdung was applied ($2tha^{-1}$) on the plot. A spacing of 0.3m x 0.3m was maintained between each seed rhizome. Sampling was carried out by harvesting four bushes (selected at random) from each bed at regular intervals of 15 days from the fourth month after planting to the end of the tenth month.

2.2 General procedure

Rhizomes (1kg to 2kg) were washed and pre-dried in the sun for 2h. All experiments were conducted separately on finger rhizome and mother sets (bulbs). Fresh rhizomes were analysed for moisture content (Dean & Stark entrainment method), oil content (Section 2.3) and oil composition (Section 2.4).

Fresh rhizomes were boiled for 10 min., sliced and dried in forced draft air oven at $60^{o}C$ for 24 h. and powdered in a micro-hammer mill. This powder was analysed for curcumins (Section 2.5).

2.3 Oil content

Fresh rhizomes were frozen at $-20^{o}C$ and ground to fine particle size using a Waring blender along with ice. The oil was extracted by water distillation using a Clavenger arm. The cooling water in the condenser was maintained at $5^{o}C$ using a Fryka–Term FT 800 cooling water circulator.

2.4 Oil composition

The volatile oil was analysed using gas liquid chromatography. Peak identification was carried out by retention data, peak enrichment and GC/MS data. Peak area normalization was used for quantification. The instrument used was a Varian 2440 instrument equipped with a Varian 9176 strip chart recorder through a Pye-Unicam DP–88 computing integrator. The operating parameters are given in Table 1. GC/MS data were recorded using a Finnigen 4000 series GC/MS system under the conditions given in Table 2. Sesquiterpene content was computed by addition of these constituents as obtained from the gas liquid chromatogram.

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Table 1 – Operating parameters for glc

Instrument model	– Varian 2440
Recorder model	– Varian 9176
Integrator model	– Pye Unicam – DP 88 (Computing integrator)
Detector	– FID
Column length	– 3 m
Column diameter	– 3mm
Liquid phase	– Carbowax 20M
Solid support	– Chromasorb W (80 – 100 mesh)
Packing material	– 10% carbowax 20M on chromasorb W
Programming	– 60 – 210°C (4°C min^{-1} and hold)
Injector temperature	– 200°C
Detector temperature	– 240°C
Carrier gas (He)	– 30 ml min^{-1}
Hydrogen supply	– 25 ml min^{-1}
Air supply	– 55 ml min^{-1}
Recorder Setting	– 1mV
Chart speed	– 5 mm min^{-1}

Table 2 – Conditions for GC/MS analysis

GC Separation	
Column length	– 2 m
Column diameter	– 2 mm
Liquid phase	– OV – 351
Solid support	– Chromosorb W (80 – 100 mesh)
Injector temperature	– 275°C
Detector	– 240°C
Programming	– 80 – 240°C (4°C min^{-1} and hold)
Carrier gas (He)	– 25 ml min^{-1}
MS details	
Ionization voltage	– 70 V
Ionization current	– 300 mA
Accelerating voltage	– 2 – 20 V
Scan-speed	– 44 AMU sec^{-1} (33 – 450)
Resolution	– 2500 at 1000 mass

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2.5 Curcumin content

Oleoresin was extracted from dried, ground turmeric (0.5g) by refluxing with 95% ethanol (100 ml) for 2.5 h. Two solutions X_1 (0.5 g 25 ml^{-1}) and X_2 (0.4 g 100 ml^{-1}) were prepared from the above solution. The curcumin content of the oleoresin was determined bv two methods both based on t.l.c. using 3 x 10^{-4} m silica gel G- 60 plates with $CHCl_3 : C_2H_5OH$ (25:1)[3] as the developing solvent.

In the first of these two methods, t.l.c.–uv spectrophotometry was used, 30 μl of solution X_1 was spotted along with 1.6 μg of individual curcumins (curcumin, demethoxycurcumin, bis-demethoxy-curcumin also known as curcumin I, II and III respectively) as standards. The curcumins were determined after extracting the yellow spot areas with C_2H_5OH by stirring the scrapings of the t.l.c. spot in a vortex mixer for 5 min (3 times). Absorbance was measured using a Varian 634 S spectrophotometer at 429, 424 and 419 nm for curcumin I, II and III respectively.

Second quantitative technique was based on t.l.c. –uv densitometry. In this technique 20 μl of solution X_2 along with 0.8 μg of each individual curcumins was spotted on the t.l.c. plate. The plate was scanned using Camag t.l.c. – hptlc variable wavelength densitometer equipped with a strip chart recorder connected to a Pye-Unicam CDPI computing integrator. Operating parameters are given in Table 3. The coefficient of variation of this method was in the range of 4.0 – 5.5%. Although there was a linear relationship between μg curcumin and peak area, a standard curve was not used for the calculation as small variations in peak area were observed from plate to plate; instead peak areas of standards (on the same plate) were used for calculations.

Table 3 – Operating parameters for t.l.c. – uv densitometry

Light source :- Tungsten		
Wave length	–	485 nm
Band width	–	30 nm
Slit width	–	12 mm
Scan speed	–	1 mm s^{-1}
Sensitivity	–	6
Recorder setting	–	500 mV
Chart speed	–	50 mm min^{-1}

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3. Results

3.1 Rhizome yield

Both experiments (in 1983/84 as well as 1984/85) yielded very similar results. Table 4 gives the effect of maturity on rhizome yield where a steady increase was observed until 9 months.

Table 4 – Effect of maturity on rhizome yield

Age (months)	Dry Weight* (g/bush)		
	M	F	Total
4	8.8	6.8	15.6
4.5	9.2	14.6	23.8
5.0	10.6	24.5	35.1
5.5	15.2	30.5	45.7
6.0	17.6	46.7	64.3
6.5	22.3	62.9	85.2
7.0	18.9	67.8	86.7
7.5	19.6	70.0	97.6
8.0	22.5	100.9	123.4
8.5	21.4	100.3	121.7
9.0	27.3	104.9	132.2
9.5	21.3	92.7	114.0
10.0	21.9	89.2	111.1

*Mean for both years

M – Mother set (bulbs)

F – Finger rhizomes

3.2 Oil content

Effect of maturity on volatile oil content is given in Table 5. Volatile oil content (% dry basis) was highest at 5 months and then declined. The oil content of the mother sets in most instances were higher than that of the fingers.

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Table 5 – Effect of maturity on volatile oil content

Age (months)	Volatile oil (ml. $100g^{-1}$, dry basis)	
	M	F
4.0	5.8	5.5
4.5	7.3	7.7
5.0	16.1	9.6
5.5	5.9	6.5
6.0	5.0	4.7
6.5	5.1	4.9
7.0	5.4	4.8
7.5	5.7	5.2
8.0	5.7	4.4
8.5	4.9	4.0
9.0	5.2	3.8
9.5	5.6	4.0
10.0	5.6	3.9

M – Mother set

F – Finger rhizome

3.3 Oil composition

A typical gas chromatogram of turmeric oil is given in Figure 1. Effect of maturity on selected essential oil components of turmeric is given in Tables 6 and 7.

Comparative study for oil from mother sets and fingers revealed that monoterpene content (α–phellandrene, 1,8 cineole and α–Terpinene) at the early maturities was higher in the fingers ($\sim$ 27%) than in the mother sets ($\sim$ 15%), while at the same time sesquiterpene content (ar-turmerone and tumerone) was higher in the mother sets. As maturity progressed these differences narrowed.

The monoterpene content of the oil from fingers declined markedly with maturity from 27% to less than 10%, while sesquiterpene content increased from 54.5 – 80%. A similar trend, to a lesser degree, was observed in the mother sets where sesquiterpene content increased from 67.5 to 84%.

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Table 6 – Effect of maturity on (selected) essential oil components (Mother Sets)

	Age (Months)												
Constituents	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
α–phellandrene	8.9	7.2	2.9	6.1	3.1	4.0	1.9	5.1	1.4	1.9	1.7	1.5	1.2
1,8–cineole	3.9	3.2	0.7	2.1	2.1	2.2	2.3	2.2	0.4	0.7	0.9	0.8	0.5
ar–turmerone	26.2	32.6	42.3	37.8	38.8	41.5	46.9	40.9	46.2	46.2	43.7	42.9	41.9
Turmerone	20.0	24.8	34.2	30.6	36.9	36.5	35.9	33.9	39.1	38.9	32.4	33.6	33.3
Sesquiterpene alcohol	8.5	7.9	8.3	7.3	5.4	4.4	3.5	1.4	2.6	1.2	3.4	3.7	4.8

Results are expressed as % total oil.

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Table 7 – Effect of maturity on (selected) essential oil components (Finger rhizomes)

Constituents	Age (Months)												
	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
α–phellandrene	15.1	13.9	15.5	11.5	14.9	14.5	12.3	10.7	9.9	9.8	3.4	6.9	1.4
1,8–cineole	6.3	5.3	3.6	2.0	4.9	2.5	3.6	2.8	1.9	2.2	1.1	1.5	0.4
ar-turmerone	23.7	24.0	39.0	39.4	36.0	42.0	37.9	39.6	38.6	39.2	39.4	40.1	36.8
Turmerone	17.6	16.7	28.7	32.6	30.6	30.8	31.4	33.0	34.5	37.1	32.9	34.7	36.6
Sesquiterpene alcohol	6.0	6.0	2.1	4.9	2.5	+	2.5	0.8	1.3	0.7	3.3	2.6	5.7

Results are expressed as % total oil

+, trace

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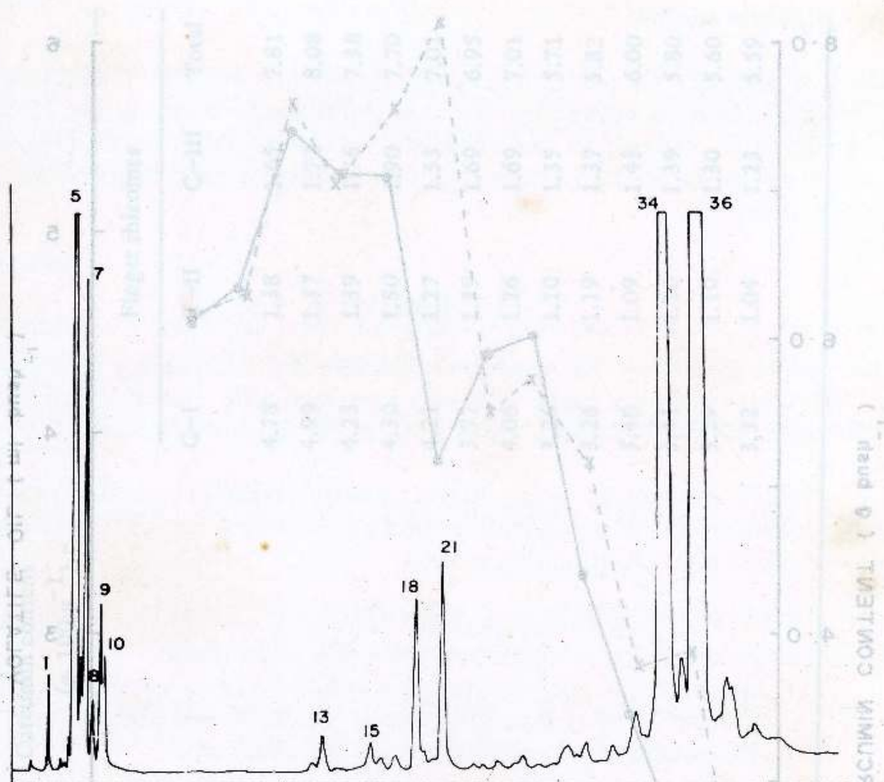

Figure 1. A TYPICAL GAS LIQUID CHROMATOGRAM OF TURMERIC OIL

1. α–pinene 5. α–phellandrene 7. 1,8 Cineole 8. γ–terpinene
9. p–cymene 10. α–terpinolene 18. Zingiberene 21. bisabolene
34. ar–turmerone 36. turmerone

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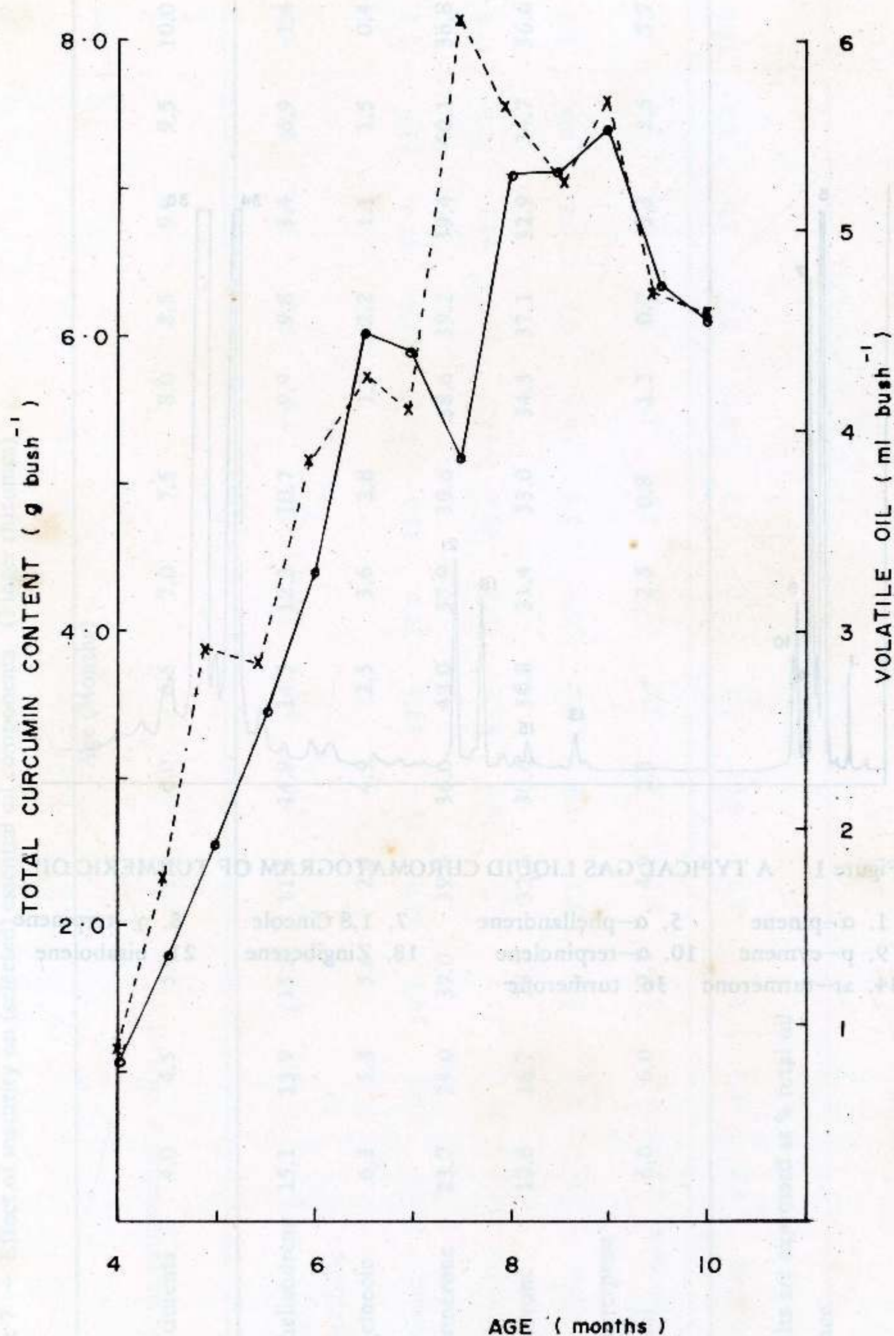

Figure 2. EFFECT OF MATURITY OF TURMERIC RHIZOME ON TOTAL OIL AND CURCUMIN PER BUSH

O———O CURCUMIN CONTENT

X- - -X OIL CONTENT

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Table 8 — Effect of maturity on curcumin content

Age (months)	Curcumin content (g. 100 g^{-1})							
	Mother rhizomes				Finger rhizomes			
	C–I	C–II	C–III	Total	C–I	C–II	C–III	Total
4.0	4.16	1.14	1.60	6.90	4.78	1.38	1.65	7.81
4.5	4.02	1.16	1.64	6.82	4.99	1.37	1.72	8.08
5.0	4.05	1.39	1.81	7.25	4.23	1.39	1.76	7.38
5.5	4.21	1.46	1.76	7.43	4.30	1.50	1.90	7.70
6.0	3.87	1.26	1.63	6.76	4.21	1.27	1.53	7.01
6.5	3.44	1.30	1.79	6.53	3.77	1.39	1.69	6.95
7.0	3.45	1.15	1.52	6.12	4.06	1.26	1.69	7.01
7.5	3.22	1.11	1.44	5.87	3.26	1.10	1.35	5.71
8.0	2.86	1.18	1.45	5.49	3.26	1.19	1.37	5.82
8.5	2.90	0.93	1.27	5.10	3.48	1.09	1.43	6.00
9.0	2.79	0.95	1.15	4.89	3.41	1.10	1.39	5.80
9.5	2.71	0.91	1.23	4.85	3.29	1.10	1.30	5.60
10.0	2.82	1.04	1.22	5.08	3.32	1.04	1.23	5.59

Results are a mean of both experimental years.

C – Curcumin

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3.4 Curcumin content

Effect of maturity on curcumin content is given in Table 8. There is decline in curcumin content both in mother sets and fingers with maturity. However total curcumin content per bush reached maximum at 9 months (Figure 2). In most instances the finger rhizomes contain higher curcumin content than mother rhizomes.

4. Discussion

When the data of both the years were pooled and analysed, it was observed that rhizome yield (dry basis) gradually increased with maturity and reached a maximum around 9 months. After this period yields declined.

Despite oil content being higher at early maturities, as the total yield per bush is low, the maximum oil content per bush is attained between 7.5 to 8.0 months after planting (Figure 2).

At the fully matured stage about 80% of the oil comprises sesquiterpenes. The results on oil composition show two main trends.

(1) Monoterpene content is higher at the outset in the finger rhizome than the mother sets. The reverse is true for sesquiterpenes.
(2) Differences narrowed as maturity was reached.

Though it is generally believed that bulbs have more colour than fingers, our results proved fingers generally contain slightly higher levels of curcumin than the mother sets (fully mature mother rhizomes contain $\sim$ 5% curcumin whereas fingers contain 5.6%).

Curcumin content reaches a maximum around 5.5 months and 4.5 to 5.0 months respectively for the mother sets and fingers thereafter declines with maturity. The total curcumin per bush reaches its highest at about 9 months after planting and this appears to be the ideal time for harvest for curcumin extraction (Figure 2).

The ratio of curcumins: demethoxycurcumin:bis–demethoxy–curcumin has been estimated previously[2,3,4,6] using t.l.c.–uv spectrophotometry. No work with t.l.c.–uv densitometer has been previously reported. In this study both methods yielded similar results.

Our results showed that curcumin I:II:III ratio does not vary to any great extent during maturation. The ratio differs from results reported from other parts of the world.[2,3,4,6]

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Acknowledgements

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3.4 Curcumin content

[illegible] In most instances the finger rhizomes contain higher curcumin content than mother rhizomes.

4. Discussion

When the data on both the years were pooled and analysed, it was observed that rhizome yield (dry basis) gradually increased with maturity and reached [illegible]

Despite oil content being higher at early maturation, as the total yield [illegible] 8.0 months after planting (Figure 3).

[illegible] The results on oil composition show two [illegible] trends.

(1) [illegible] content is higher at the outset in the finger rhizome than the mother sets. [illegible]

(2) Differences narrowed as maturity was reached.

Though it is generally believed that bulbs have more colour than fingers, our results proved fingers generally contain slightly higher levels of curcumin than the mother sets (fully mature mother rhizomes contain [illegible] curcumin whereas fingers contain [illegible]).

Curcumin content reaches a maximum around 6.5 months and [illegible] to 5.0 months respectively for the mother sets and fingers thereafter declines with maturity. The total curcumin per bush reaches its highest at about [illegible] months after planting and this appears to be the ideal time for harvest for curcumin extraction (Figure 2).

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Our results showed that curcumin I:II:III ratio does not vary to any great extent during maturation. The ratio differs from results reported from other parts of the world.[illegible]

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STUDIES ON THE PRODUCTION OF PLASTER OF PARIS FROM DISCARDED MOULDS

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Abstract : Investigations have been made to develop a simple method to convert discarded moulds in ceramic industry to Plaster of Paris. With this in view, physical and chemical properties of used moulds, Plaster of Paris, gypsum and the products obtained by firing used moulds under different conditions have been determined. Monoclinic form of $CaSO_4.2H_2O$ has been identified in the used moulds while Plaster of Paris contains β form of $CaSO_4.½H_2O$. DTA and XRD analysis show that a temperature lower than 350°C but higher than 170°C is required for the conversion. Rapid heating rates are required for the formation of $\beta-CaSO_4.½H_2O$ which gives soluble anhydrite at 170°C. At temperatures higher than 350°C, soluble anhydrite is transformed into an inactive form. Good quality Plaster of Paris could be produced by heating powdered moulds at 180°C for 2 hours in an open atmosphere. Breaking strength, chemical composition and other properties of the product thus obtained conform to the standard requirements of commercial Plaster of Paris.

1. Introduction

Plaster of Paris is produced by calcining gypsum. The main deposits of gypsum are found in United States, France, West Germany, Poland and Austria. A large quantity of the world production of Plaster of Paris is used in the ceramic industry for the manufacture of moulds and as a cementitious material for construction purposes.[2] It is also used for the manufacture of wall boards and for dental and orthopaedic work.[2]

In Sri Lanka, plaster of Paris is mainly used for making tableware and sanitaryware moulds in the ceramic industry and as a binding material for plastering purposes. In 1986, the total production of moulds at the Ceylon Ceramics Corporation, Piliyandala, was around 32–35 metric tons per month. After using the moulds for a certain period, these are mainly discarded as a waste material. Some of these have been used for the manufacture of writing chalk and for land filling. Plaster of Paris is imported to

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Sri Lanka at a very high cost and therefore, it is important to study the possibility of recycling the Plaster of Paris. In order to develop a method of recycling, it is essential to study the physical and chemical properties of used moulds and Plaster of Paris.

In the present study, investigations have been made in the laboratory scale, to determine the most suitable conditions of regenerating Plaster of Paris from used moulds.

2. Experimental

Used moulds were ground to pass the sieve (300 μm, mesh No.50) and burnt under two different conditions, (i) in a muffle furnace and (ii) in a special vessel (Figure 1) using burners. The constituents in used moulds, Plaster of Paris and the fired products were determined by powder X-ray diffraction with Cu K_{α} radiation. Thermal analyses (DTA, TG and DTG) of used moulds, gypsum, Plaster of Paris and the fired products were done using a Thermal Analyzer model NETZSCH STA 409. Transverse breaking strengths have been determined by a standard procedure.[5]

Chemical analyses of used moulds and the fired product for CaO, Al_2O_3, SO_3 were done using gravimetric methods.[1] Fe_2O_3 was determined by a titrimetric method.[1] Na_2O and K_2O were estimated by using a Flame Photometer and MgO was determined by using an atomic absorption spectrometer. SiO_2 was determined by the HF treatment.[3]

3. Results and Discussion

3.1 Physical Properties of used moulds and Plaster of Paris

3.1.1 X-ray Diffraction Analysis

Powder X-ray diffraction patterns of crockery and sanitaryware moulds showed the presence of $CaSO_4.2H_2O$ in the monoclinic form. Other compounds were not found in detectable quantities. Imported Plaster of Paris showed the presence of hexagonal type of $CaSO_4.O.5H_2O$. XRD patterns of used moulds and Plaster of Paris are given in Figure 2.

3.1.2 Thermal Analysis

Thermal analyses of used moulds, gypsum and Plaster of Paris were carried out under different conditions and the DTA curves are given in Figure 3. DTA curve of gypsum showed two significant endothermic peaks. The large

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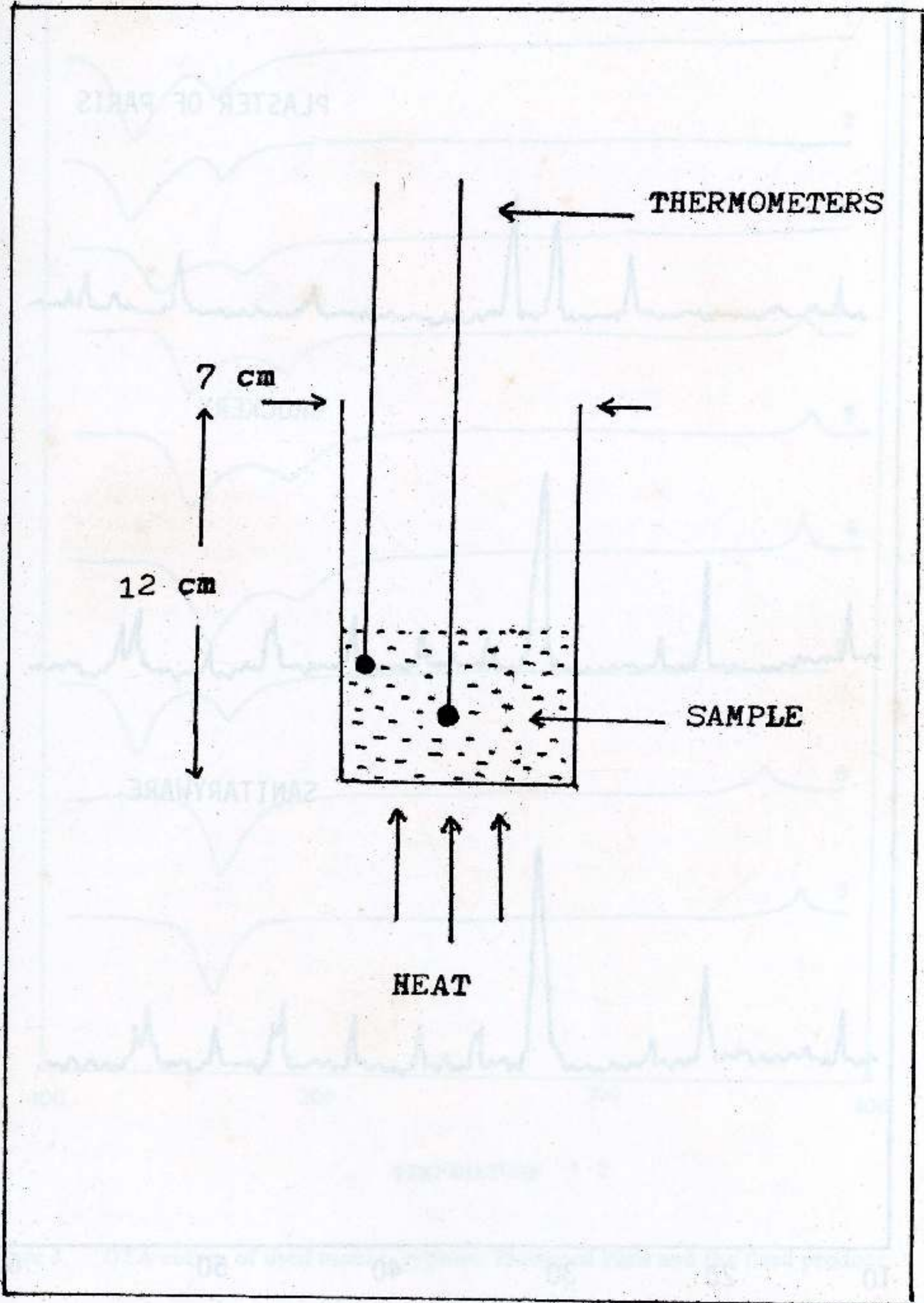

Figure 1. Vessel used for burning used moulds

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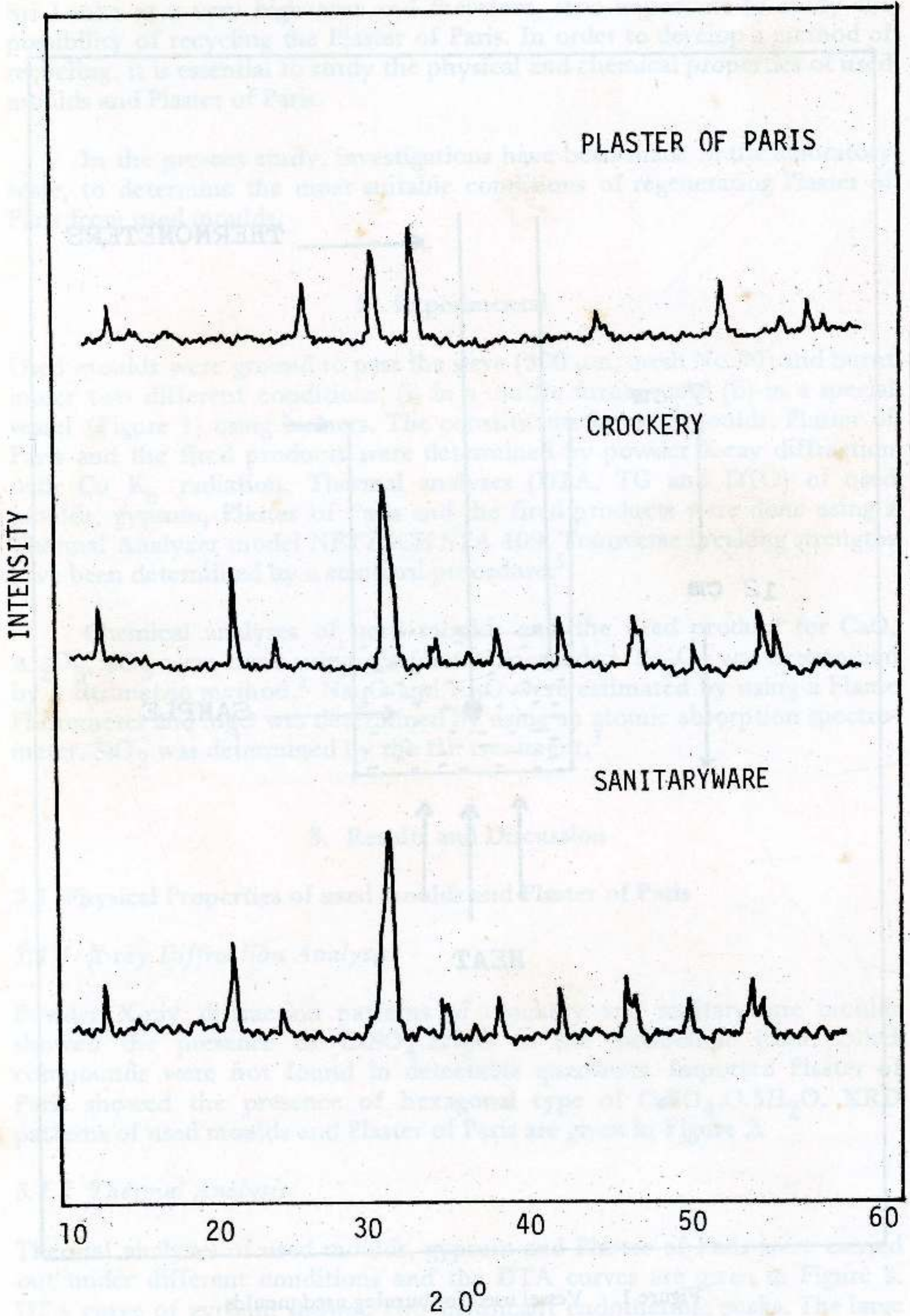

Figure 2. XRD patterns of used mould and Plaster of Paris

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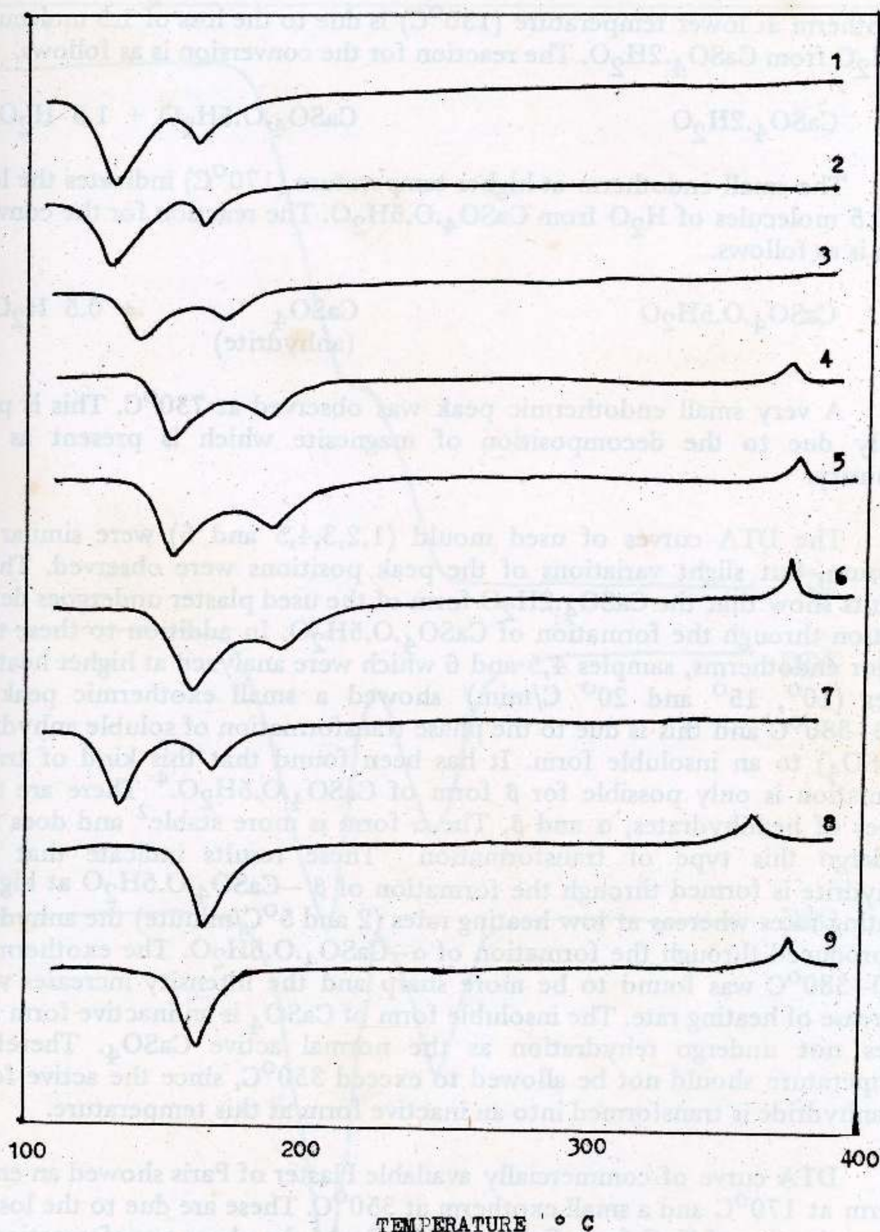

Figure 3. DTA curves of used mould, gypsum, Plaster of Paris and the fired product.

N.B. 1,3,4,5 and 6 are thermograms of used mould at heating rates 2°, 5°, 10°, 15° and 20°C per minute respectively. No.2 is a thermogram taken at 2°C per minute of a sample of used mould which has been preheated at 45°C for 4 hrs. 7,8 and 9 are thermograms for gypsum, Plaster of Paris and the fired product at the heating rate of 2°C per minute, respectively.

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endotherm at lower temperature (130°C) is due to the loss of 1.5 molecules of H_2O from $CaSO_4.2H_2O$. The reaction for the conversion is as follows.

$$CaSO_4.2H_2O \qquad CaSO_4.O.5H_2O + 1.5\ H_2O$$

The small endotherm at higher temperature (170°C) indicates the loss of 0.5 molecules of H_2O from $CaSO_4.O.5H_2O$. The reaction for the conversion is as follows.

$$CaSO_4.O.5H_2O \qquad \underset{\text{(anhydrite)}}{CaSO_4} + 0.5\ H_2O$$

A very small endothermic peak was observed at 730°C. This is probably due to the decomposition of magnesite which is present as an impurity.

The DTA curves of used mould (1,2,3,4,5 and 6) were similar to gypsum, but slight variations of the peak positions were observed. These results show that the $CaSO_4.2H_2O$ form of the used plaster undergoes dehydration through the formation of $CaSO_4.O.5H_2O$. In addition to these two major endotherms, samples 4,5 and 6 which were analyzed at higher heating rates (10°, 15° and 20° C/min.) showed a small exothermic peak at 350–380°C and this is due to the phase transformation of soluble anhydrite ($CaSO_4$) to an insoluble form. It has been found that this kind of transformation is only possible for β form of $CaSO_4.O.5H_2O$.[4] There are two types of hemihydrates, α and β. The α form is more stable[2] and does not undergo this type of transformation. These results indicate that the anhydrite is formed through the formation of β –$CaSO_4.O.5H_2O$ at higher heating rates whereas at low heating rates (2 and 5°C/minute) the anhydrite is produced through the formation of α–$CaSO_4.O.5H_2O$. The exotherm at 350–380°C was found to be more sharp and the intensity increases with increase of heating rate. The insoluble form of $CaSO_4$ is an inactive form and does not undergo rehydration as the normal active $CaSO_4$. Therefore temperature should not be allowed to exceed 350°C, since the active form of anhydride is transformed into an inactive form at this temperature.

DTA curve of commercially available Plaster of Paris showed an endotherm at 170°C and a small exotherm at 350°C. These are due to the loss of 0.5 molecules of H_2O from $CaSO_4.O.5H_2O$ and the phase transformation of soluble anhydrite to an insoluble form. This result indicates that the commercial type of Plaster of Paris constitutes β –$CaSO_4.O.5H_2O$.

TG curve of the used moulds showed one large and one small weight loss and these are due to the loss of 1.5 and 0.5 molecules of H_2O respectively. DTG curve also showed two sharp peaks due to these two weight losses. Figure 4 shows TG, DTG and DTA curves of the used moulds.

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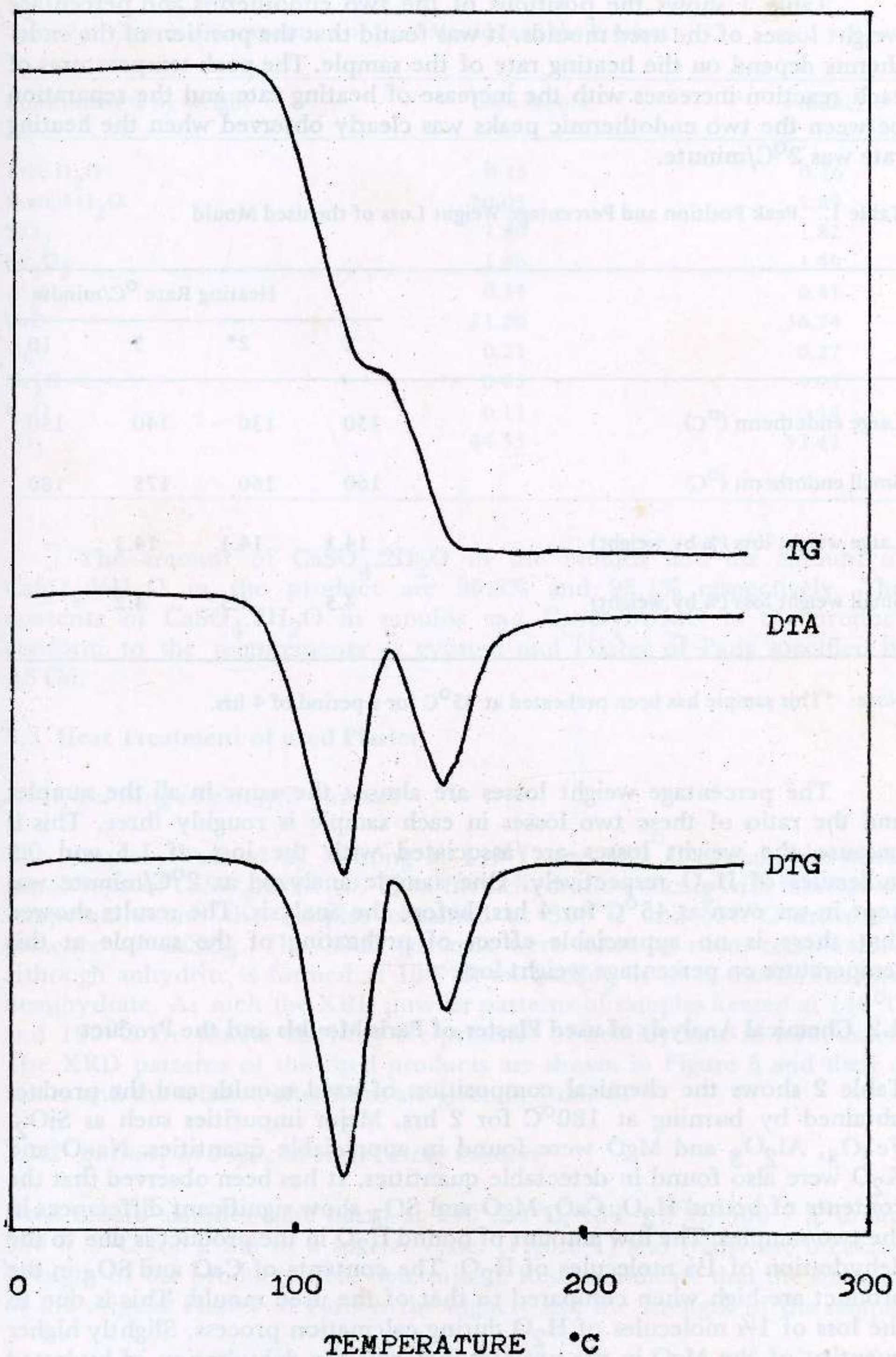

Figure 4. TG, DTG and DTA curves of used moulds

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Table 1 shows the positions of the two endotherms and percentage weight losses of the used moulds. It was found that the position of the endotherms depend on the heating rate of the sample. The peak temperatures of each reaction increases with the increase of heating rate and the separation between the two endothermic peaks was clearly observed when the heating rate was $2^{o}C$/minute.

Table 1. Peak Position and Percentage Weight Loss of the used Mould

	Heating Rate ^{o}C/minute			
	2	2*	5	10
Large endotherm (^{o}C)	130	130	140	150
Small endotherm (^{o}C)	160	160	175	180
Large weight loss (% by weight)	14.1	14.1	14.3	–
Small weight loss (% by weight)	4.5	4.5	4.2	–

Note: *This sample has been preheated at $45^{o}C$ for a period of 4 hrs.

The percentage weight losses are almost the same in all the samples and the ratio of these two losses in each sample is roughly three. This is because the weight losses are associated with the loss of 1.5 and 0.5 molecules of H_2O respectively. One sample analyzed at $2^{o}C$/minute was kept in an oven at $45^{o}C$ for 4 hrs. before the analysis. The results showed that there is no appreciable effect of preheating of the sample at this temperature on percentage weight loss.

3.2 Chemical Analysis of used Plaster of Paris Moulds and the Product

Table 2 shows the chemical composition of used moulds and the product obtained by burning at $180^{o}C$ for 2 hrs. Major impurities such as SiO_2, Fe_2O_3, Al_2O_3 and MgO were found in appreciable quantities. Na_2O and K_2O were also found in detectable quantities. It has been observed that the contents of bound H_2O, CaO, MgO and SO_3 show significant differences in the two samples. The low amount of bound H_2O in the product is due to the dehydration of 1½ molecules of H_2O. The contents of CaO and SO_3 in the product are high when compared to that of the used mould. This is due to the loss of 1½ molecules of H_2O during calcination process. Slightly higher quantity of the MgO in the product indicates the dehydration of hydrated MgO during thermal treatment.

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Table 2. Chemical Composition of Used Moulds and the Product

Constituent % by weight	Used Mould	Product
Free H_2O	0.15	0.16
Bound H_2O	20.05	5.89
SiO_2	1.80	1.82
Fe_2O_3	1.46	1.49
Al_2O_3	0.34	0.41
CaO	31.20	36.74
MgO	0.21	0.27
Na_2O	0.05	0.05
K_2O	0.11	0.14
SO_3	44.55	52.43

The amount of $CaSO_4.2H_2O$ in the moulds and the amount of $CaSO_4.½H_2O$ in the product are 95.8% and 95.1% respectively. The contents of $CaSO_4.2H_2O$ in moulds and $CaSO_4.½H_2O$ in the product conform to the requirements of gypsum and Plaster of Paris specified in ASTM.

3.3 Heat Treatment of used Plaster

3.3.1 Burning in a muffle furnace

The samples obtained by burning at 140°C and 180°C contain the hexagonol type of $CaSO_4.O.5H_2O$. Then the dehydration occurs with increase of temperature and the samples obtained at 300°C and 550°C show the presence of $CaSO_4$. The XRD patterns were taken at room temperature although anhydrite is formed at 180°C, on cooling in air it transforms into hemihydrate. As such the XRD powder patterns of samples heated at 140°C and 180°C are similar showing the presence of hemidydrate in both cases. The XRD patterns of the fired products are shown in Figure 5 and their *d* values and the relative intensities are given in Table 3.

3.3.2 Burning in a special vessel using burners

Used mould samples were burnt at 140 ± 3°C, 180 ± 3°C, 300 ± 3°C and 550 ± 3°C for 2 hrs in a special vessel (Figure 1) and transverse breaking strength of the products were determined. Results indicate that the strength of the original Plaster of Paris is the highest and the strength of the sample

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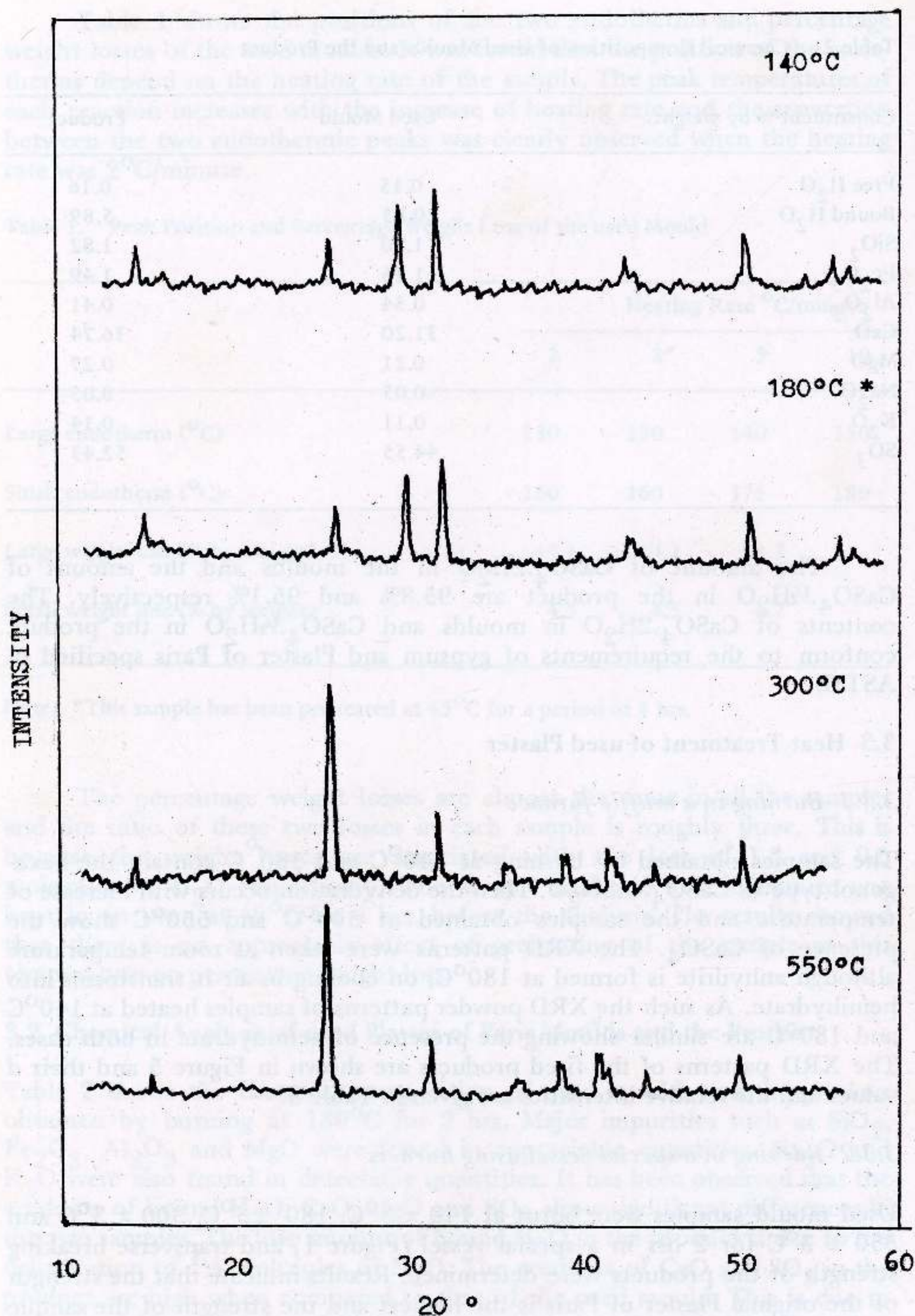

Figure 5. XRD patterns of products obtained by burning used moulds

N.B. * gives the most suitable burning condition

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Table 3. X-ray Diffraction Data of Fired Samples

140°C/2 hrs.		180°C/2 hrs.		300°C/2 hrs.		550°C/2 hrs.	
d(A°)	I	d(A°)	I	d(A°)	I	d(A°)	I
6.10	57	6.15	57	6.71	17	6.70	17
3.51	60	3.52	60	6.55	15	6.55	15
3.04	90	3.04	92	3.48	100	3.48	100
2.85	100	2.85	100	2.83	33	2.83	33
2.12	32	2.15	32	2.43	11	2.45	12
1.86	60	1.86	60	2.32	20	2.32	20
1.72	32	1.70	33	2.18	23	2.19	24
1.65	22	1.67	22	2.17	17	2.17	17
				2.05	15	2.07	13
				1.86	20	1.86	21
				1.74	17	1.74	17

obtained at 180°C is very close to that of Plaster of Paris. Therefore this result shows that the most suitable temperature of burning used plaster to obtain Plaster of Paris is 180°C. The strength increases upto 180°C and decreases with increase of temperature. It is significant that there is a sudden drop of strength when temperature rises from 300°C to 550°C. This is because the active form of $CaSO_4$ converts to an inactive form during this temperature range as shown in DTA. Table 4 shows the breaking strength of the products.

Table 4. Breaking Strength of the Products and Plaster of Paris

Sample	Temperature (oC)	Duration (hrs.)	Breaking strength (grams)
Calcined product	140	2	267
Calcined product	180	2	375
Calcined product	300	2	322
Calcined product	550	2	117
Plaster of Paris			392

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Used mould samples were also burnt at 180 ± 3°C for 1,2,3 and 4 hrs and breaking strength of the products were determined. It was found that the breaking strength of the product increases with increase of duration of burning upto 2 hrs, and remains constant. It has been observed that there is no appreciable effect of further burning beyond 2 hrs, on the quality of the product. These results indicate that the most suitable duration of burning of used moulds is 2 hrs. Breaking strength of the products are given in Table 5.

Table 5. Breaking Strength of the Products obtained by Burning Used Mould at 180°C for Different Durations

Duration of Burning (hrs.)	Breaking Strength (grams)
1	280
2	375
3	375
4	370

3.4 Properties of the Fired Product

Powder XRD pattern of the product obtained by burning used moulds at 180°C for 2 hours in an open atmosphere is almost similar to that of Plaster of Paris. DTA of the product shows an endotherm at 170°C and an exotherm at 380°C. These two peaks in DTA are due to the loss of $\frac{1}{2}H_2O$ from β $CaSO_4.\frac{1}{2}H_2O$ to form anhydrous $CaSO_4$ and the transformation of soluble anhydrite to the insoluble form respectively. Relevant DTA curves are shown in Figure 3 while the XRD pattern of the product is given in Figure 5.

It is apparent that the material produced by calcination of the used moulds at 180°C consists mostly of the hemihydrate. The calcination temperature 180°C is however higher than the dehydration temperature of hemihydrate. Therefore the product of calcination at 180°C is probably the soluble anhydrite, which on cooling and exposure to air forms the hemihydrate.

Breaking strengths of the products obtained by burning the used moulds at different temperatures are compared with the breaking strengths of Plaster of Paris in Table 4. The transverse breaking strength of the product obtained at 180°C (375g) is very similar to that of Plaster of Paris (392 g). The products obtained at other temperatures have much lower breaking strengths. Thus, it is clear that the optimum temperature of conversion is about 180°C.

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4. Conclusions

Plaster of Paris can be produced by the heat treatment of discarded moulds under different conditions and the quality of the product thus obtained depends on the temperature and duration of burning. No appreciable differences have been observed in the products obtained by the two methods of burning used in the present study. Burning in a kiln requires high expenditure and as such open burning method seems to be more economical for a commercial process. DTA studies have shown that a temperature higher than 140°C and a rapid heating rate are required for the formations of β–$CaSO_4.0.5H_2O$ which undergoes further dehydration at 170°C to give an active form of anhydrite. Since the active form of $CaSO_4$ is transformed into inactive form at about 350°C, the burning temperature should be controlled below 350°C.

A temperature of 180°C and a heating duration of 2 hours are found to be most suitable for the conversion of used moulds to Plaster of Paris. Physical and chemical properties of the product thus obtained are very similar to those of commercial Plaster of Paris. Therefore, the product obtained under these conditions can be used for the manufacture of new moulds and as a cementitious material for construction purposes.

Acknowledgements

The authors wish to thank the Director and Deputy Directors of the Ceylon Institute of Scientific and Industrial Research and the staff of the Minerals Technology Section of CISIR for their cooperation. We are grateful to Professor D.T.B. Tennakoon for critical review of the manuscript.

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BODY SIZE DATA OF SRI LANKAN WORKERS AND COMPARISON WITH OTHER POPULATIONS IN THE WORLD: ITS IMPACT ON THE USE OF IMPORTED GOODS

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Abstract : A national anthropometric survey of Sri Lankan workers has provided uptodate information on the body sizes of both males and females. This data (which was lacking in Sri Lanka) can be of great benefit for designers and importers of goods for Sri Lankans. Simple and anthropologically spread techniques have been used to collect the data. Comparisons of body sizes between developing countries (including Sri Lanka) with those of developed countries revealed wide variations which have been responsible in the incompatibility in the use of imported goods. Anthropometric data of homogenous populations in different countries are not usually available and more so in developing countries. It is emphasized that such data be developed in all countries or in every ethnic group or on a regional basis. The designers, manufacturers and importers of goods should have access to this data. It is suggested that the techniques and methods described in this paper can be used by developing countries lacking similar data.

1. Introduction

Sri Lanka imports machinery, vehicles and various other items and articles manufactured in foreign countries. These imported items which are manufactured to fit people in those countries, in many instances, cannot be used safely, efficiently and comfortably by the local population. Many problems of inadequacies in design have been experienced by Sri Lankans, either in using imported and even locally produced machines, which have been made disregarding the body sizes of the users. In order to design machines, articles, garments and work places to match a population, an important requirement for the designer is the information regarding body dimensions of the people. There are records of anthropometry of Sri Lankans, but they refer to specific age groups or populations viz. University students[3] or to school children[5] and more recent data collected with the purpose of ascertaining the physical differentiation of the main races in Sri Lanka.[13] As the required body measurements of the homogenous population of Sri Lanka are not available, a survey was planned and carried out on a national sample of the population of workers, both male and female. The techniques and procedures described in this paper can be of benefit to those planning such surveys.

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Sri Lanka is a tropical Asian country with a geographical area of approximately 65,000 square kilometers and a population of about 16 million people of whom about 30% consists of the working population. Workers were taken as subjects for the body size survey as it was easy to reach them, for measuring convenience of random sampling and they represented a homogenous population. Other reasons are that the majority of imported goods and machines are used by workers. In Industrially Developing Countries (IDC) including Sri Lanka, the unavailability of body size data as well as unawareness of the importance of ergonomics principles are the main reasons why machines and goods are not manufactured or adapted to suit the user population. In this paper anthropometric data of Sri Lankans and other IDC are compared with data of Industrialized Countries (IC) to show the variability in body sizes that exist. The design incompatibility in goods seems to be an important aspect of the problems involved in the transfer of technology from developed to developing countries.

2. Methods and Procedures

The national anthropometric survey of workers in Sri Lanka was carried out during a period of 16 months in 1981/82. The measurements were taken by three trained investigators. A pilot anthropometric survey lasting for three weeks was conducted on 100 fibre mill workers which provided the investigators with adequate experience in carrying out body measurements accurately and speedily.

A total of 90 body measurements, which were chosen to cover anthropometric informations for designers[2,8] were taken. The total number of subjects was 724 between the ages of 21 and 51 years, of which 438 (60.5%) were males with mean weight 51 kg, (S.D. 8.3 kg), and 286 (39.5%) were females with a mean weight 43 kg, (S.D. 6.5 kg). This sample included workers from all 24 districts from the 7 provinces of Sri Lanka, (Figure 1). The number of subjects from each district was selected on a proportional basis in accordance with the employment survey statistics of 1974. The five main races which represented the national sample consisted of 581 (80%) Sinhalese, 78 (11%) Indian Tamils, 53 (7.5%) Ceylon Tamils, 9 (1%) Moors and 3 (0.5%) Burghers. Members of the main religious groups, communities and armed forces and also veddhas (a jungle tribe) have been represented in the sample. The subjects from 80 work establishments and employed in various categories of work were selected at random. Except for the age, sex, race and number of subjects from each district, there was no discrimination of the selection of subjects. Sick and disabled persons were excluded.

Simple but accurate measuring instruments were used considering the requirements of easy transportation, reliability of results, durability and

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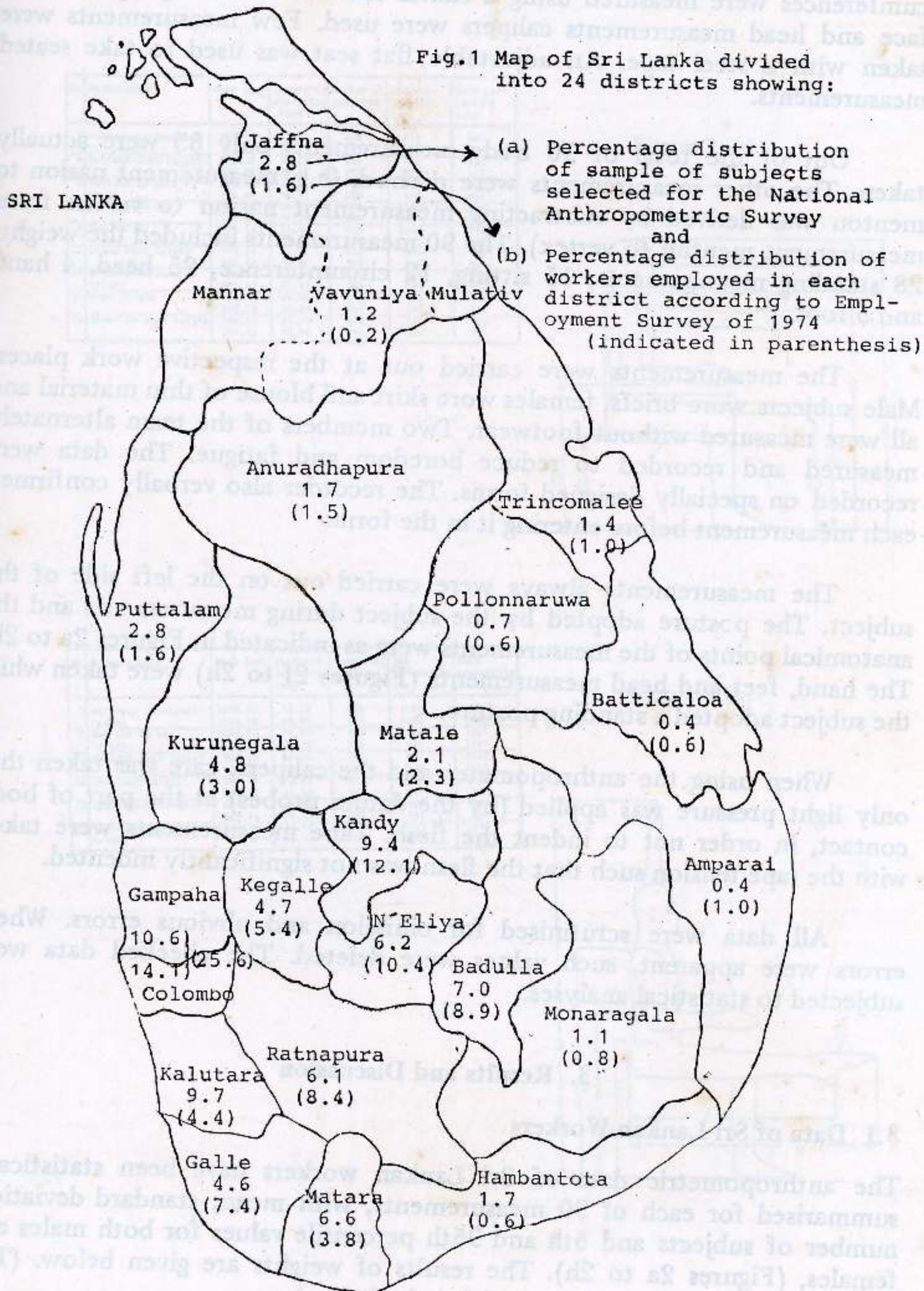

Fig.1 Map of Sri Lanka divided into 24 districts showing:

(a) Percentage distribution of sample of subjects selected for the National Anthropometric Survey and

(b) Percentage distribution of workers employed in each district according to Employment Survey of 1974 (indicated in parenthesis)

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speed of operation and use. The main instrument, Harpenden anthropometer was used to take 50% of the measurements (linear measurements). The circumferences were measured using a canvas flexible measuring tape and for face and head measurements calipers were used. Few measurements were taken with a steel tape. An adjustable flat seat was used to take seated measurements.

Out of the total of 90 body measurements only 85 were actually taken. The other measurements were derived, (e.g. measurement nasion to menton was derived by subtracting measurement nasion to vertex from measurement menton to vertex). The 90 measurements included the weight, 28 standing measurements, 15 sitting, 12 circumference, 25 head, 4 hand and 5 foot.

The measurements were carried out at the respective work places. Male subjects wore briefs, females wore skirt and blouse of thin material and all were measured without footwear. Two members of the team alternately measured and recorded to reduce boredom and fatigue. The data were recorded on specially designed forms. The recorder also verbally confirmed each measurement before entering it in the form.

The measurements always were carried out on the left side of the subject. The posture adopted by the subject during measurements and the anatomical points of the measurements were as indicated in Figures 2a to 2h. The hand, feet and head measurements (Figures 2f to 2h) were taken while the subject adopted a standing posture.

When using the anthropometer and the calipers, care was taken that only light pressure was applied (by the datum probes) at the part of body contact, in order not to indent the flesh. Tape measurements were taken with the tape tension such that the flesh was not significantly indented.

All data were scrutinised for omission and obvious errors. Where errors were apparent, such values were deleted. The checked data were subjected to statistical analyses.

3. Results and Discussion

3.1 Data of Sri Lankan Workers

The anthropometric data of Sri Lankan workers have been statistically summarised for each of 90 measurements, with mean, standard deviation, number of subjects and 5th and 95th percentile values for both males and females, (Figures 2a to 2h). The results of weights are given below. (The results for males appear above and females below).

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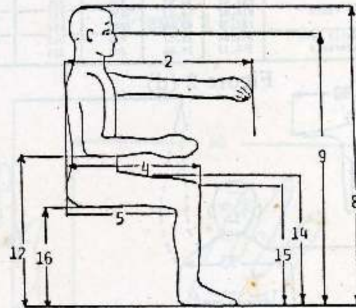
SITTING MEASUREMENTS

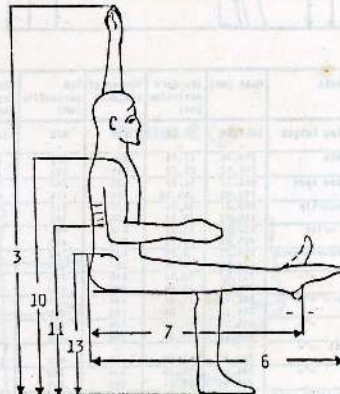
No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
2	Horizontal functional reach	780.79 724.49	43.91 76.27	436 287	712 653	864 790
4	Buttock-Knee Length	656.36 534.22	32.27 29.49	436 288	611 487	620 572
6	Buttock to back of knee	459.19 445.67	30.65 35.41	436 288	416 400	507 494
8	Sitting height	1213.68 1123.15	66.63 44.39	434 288	1130 1055	1296 1190
9	Eye height	1108.90 1022.69	51.34 45.98	435 287	1031 940	1195 1090
12	Elbow rest height	578.39 534.42	51.81 39.46	436 287	509 470	663 600
14	Thigh clearance height	500.57 436.42	25.44 23.99	435 288	460 424	548 503
15	Knee height	456.32 422.10	28.09 26.89	431 284	410 374	502 464
16	Stool or seat height	380.14 349.31	29.05 24.16	434 285	338 316	423 341

Figure 2 (a)



Nu	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
3	vertical functional reach	1640.07 1490.56	75.85 68.65	435 286	1514 1386	1756 1615
6	Buttock to toe length	1099.50 1033.36	71.44 78.78	433 286	1002 947	1202 1127
7	Buttock heel length	988.50 926.25	61.90 63.57	436 287	904 843	1070 1009
10	Shoulder height	949.34 873.17	50.61 44.96	434 288	876 811	1025 940
11	Lowest rib height	644.76 607.16	44.63 36.55	435 288	585 550	715 669
13	Upper hip bone height	543.95 510.32	42.52 36.32	436 287	465 457	619 571

Figure 2 (b)



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STANDING MEASUREMENTS

Figure 2 (c)

No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
17	Stature	1639.01 1522.77	63.61 58.77	435 288	1536 1426	1746 1617
18	Nasion height	1555.19 1439.50	62.07 59.56	435 288	1448 1343	1659 1539
19	Eye height	1535.31 1418.89	62.19 58.87	432 288	1429 1323	1638 1520
20	Subnasale height	1503.25 1398.70	61.20 58.46	435 287	1403 1295	1601 1488
21	Menton height	1435.29 1327.46	59.32 56.72	435 287	1342 1272	1537 1424
22	Tragion height	1507.72 1396.68	60.46 57.69	434 289	1412 1304	1607 1490
24	Elbow height	1014.04 941.46	70.04 62.11	436 286	929 873	1100 1016
31	Chest depth	170.60 161.53	19.67 19.40	436 288	146 136	204 193
38	Elbow wrist length	276.31 251.91	34.64 33.98	435 288	247 225	302 276
39	Forward reach	817.02 757.37	59.83 46.40	436 288	747 692	807 828
57	Knee fully bent circumference	391.68 355.77	25.57 24.86	435 285	350 320	430 395

Figure 2 (d)

No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
23	Shoulder height	1377.09 1272.61	59.24 53.99	435 287	1280 1184	1473 1360
25	Waist height	1068.24 1012.79	64.35 57.05	435 288	976 931	1160 1084
27	Hip height	971.91 920.71	58.51 75.94	434 286	885 840	1062 987
28	Crotch height	775.32 776.24	48.91 60.90	433 186	707 690	841 846
29	Finger tip height	607.16 571.28	38.77 45.11	435 288	554 504	669 626
30	Span	1690.56 1544.66	88.64 98.93	435 288	1566 1407	1816 1678
32	Chest breadth	249.35 224.77	26.80 19.08	436 289	216 199	283 252
33	Biacromial breadth	368.29 331.40	23.40 18.46	434 288	331 300	402 361
40	Upward reach	2083.74 1912.78	115.19 118.59	435 288	1947 1775	2236 2051
41	Ankle circumference	196.01 182.18	14.84 11.83	432 288	175 165	220 205
42	Calf -"-	306.14 290.48	27.40 22.80	433 286	265 252	350 325
43	Thigh -"-	459.24 456.97	48.54 46.98	434 234	395 390	545 540
48	Buttock -"-	823.25 832.92	56.55 53.97	432 287	740 750	930 925
49	Waist -"-	688.63 633.44	68.16 61.24	436 288	605 555	800 740
50	Abdomen -"-	717.42 727.16	70.39 71.76	436 288	630 620	855 860

Figure 2 (e)

No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
26	Outside leg length	1053.60 -	74.14 -	258 -	970 -	1140 -
34	Hip breadth	250.36 246.42	17.74 22.22	436 285	224 212	280 281
35	Inter elbow span	861.12 793.81	51.29 105.98	435 288	782 718	940 868
36	Elbow-fingertip length	450.37 410.32	34.26 29.32	435 289	414 378	492 449
37	Acromial wrist length	532.15 495.91	27.22 29.71	436 288	486 450	573 542
44	Wrist circumference	154.32 138.66	9.79 8.65	435 287	140 125	170 150
46	Fore arm -"-	233.07 204.58	20.62 15.78	435 288	205 180	265 235
47	Upper arm -"-	242.55 224.11	24.87 27.09	435 288	205 190	290 260
51	Chest -"-	805.80 808.11	55.03 58.35	436 288	725 720	915 905
52	Under bust -"-	- 724.30	- 62.68	- 178	- 620	- 1060
53	Neck -"-	341.62 294.86	22.20 19.67	436 288	310 265	380 330
54	Waist to waist over shoulder	752.89 680.26	43.01 45.63	435 284	680 610	825 750
55	Crotch length	736.02 -	66.99 -	430 -	650 -	840 -
56	Elbow fully bent circumference	298.34 260.26	27.58 21.32	432 287	260 230	340 295

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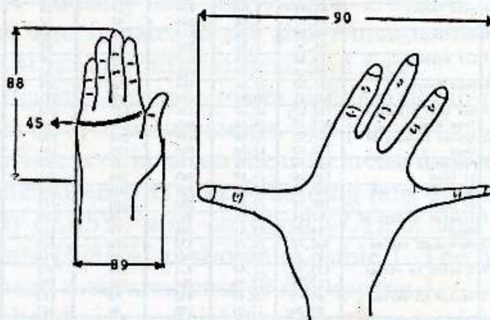
HAND AND FOOT MEASUREMENTS

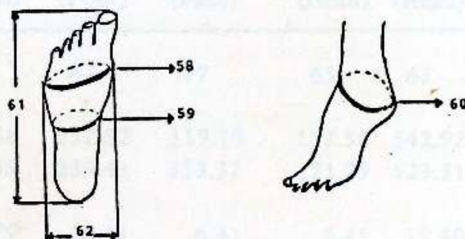
No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
45	Hand girth	196.19	15.75	260	175	220
		176.57	13.21	182	160	190
88	Hand Length	179.38	12.03	435	165	195
		166.59	14.02	287.	150	182
89	Hand breadth	99.50	6.53	435	90	110
		88.69	5.59	287	80	99
90	Finger span	207.64	15.19	435	185	232
		184.31	15.82	286	160	210

Figure 2 (f)



No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
58	Ball of foot circumference	228.80	16.42	433	208	250
		206.39	13.41	288	185	230
59	Inspet sole circumference	235.95	15.42	434	215	260
		210.73	14.27	288	190	235
60	Heel Instep circumference	319.00	17.96	434	290	345
		289.06	16.47	288	265	315
61	Foot length	252.82	17.43	434	230	280
		234.41	13.15	288	210	255
62	Foot breadth	105.76	10.63	435	90	120
		95.70	12.08	288	80	110

Figure 2 (g)



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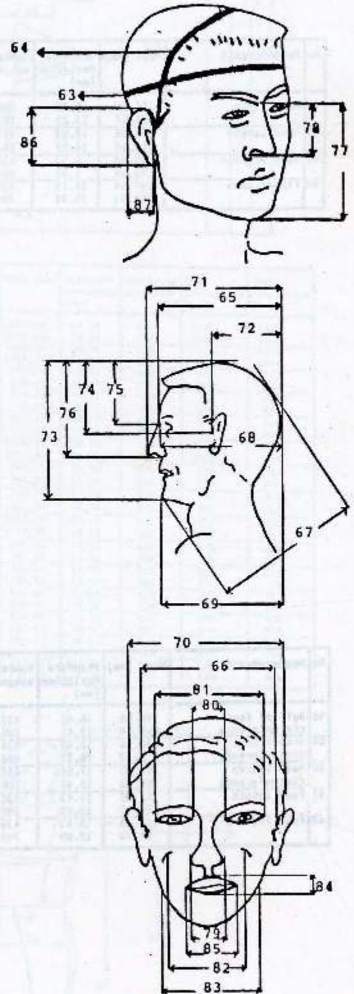
HEAD AND FACE MEASUREMENTS

Figure 2 (h)

No	Measurements	Mean (mm)	Standard deviation (mm)	Number of subjects	5th percentile (mm)	95th percentile (mm)
63	Head circumference	542.91 523.51	18.42 14.61	436 288	515 500	570 550
64	Bitragion coronal arc	343.21 327.46	23.68 23.50	436 288	320 300	370 355
77	Nasion to menton	119.78 113.37	17.53 16.10	431 287	97 89	144 135
78	Nose length	51.95 49.57	5.04 5.56	435 288	44 42	59 57
86	Ear length	59.09 55.35	4.90 5.38	433 286	52 47	67 62
87	Ear breadth	29.24 27.68	3.16 3.79	433 286	25 23	34 32
65	Head length	177.50 171.25	12.06 20.43	434 288	156 149	194 191
67	Max head diagonal from menton	238.87 227.33	10.37 14.79	436 285	222 214	253 241
68	Nasion to back of head	177.52 170.53	12.14 12.88	434 288	160 150	196 191
69	Menton to back of head	196.40 186.18	16.56 15.69	434 288	173 160	224 211
71	Nose tip to back of head	207.52 198.78	16.02 12.68	434 288	188 180	226 219
72	Tragion to back of head	97.14 94.21	13.91 13.41	431 286	77 74	118 117
73	Menton to vertex	202.72 193.38	28.76 20.31	432 287	169 163	234 218
74	Tragion to vertex	133.78 127.14	21.52 13.99	431 288	113 106	153 146
75	Nasion to vertex	85.72 84.25	20.41 17.09	432 288	59 56	110 107
76	Subnasale to vertex	136.81 134.42	22.75 17.05	432 287	111 107	165 160
66	Bitragion diameter	129.61 122.17	20.18 12.68	435 288	118 110	140 131
70	Head breadth	142.75 136.85	11.66 17.32	436 288	130 124	153 146
79	Nose breadth	37.26 34.02	4.50 4.16	435 288	32 29	44 40
80	Interocular breadth	30.47 29.35	4.39 2.95	435 288	25 24	35 34
81	Biocular breadth	103.08 99.40	6.60 8.69	435 288	93 87	114 110
82	Bizygumetic breadth	101.91 99.05	8.32 9.33	433 288	87 85	115 114
83	Bigonial breadth	91.79 87.49	11.91 12.19	433 288	76 74	108 102
84	Mouth height	21.11 20.25	3.24 2.69	435 288	16 16	26 24
85	Mouth length	52.10 49.84	4.40 4.35	435 288	44 43	59 56



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No	Measurement	Mean (kg)	Standard Deviation	Number of Subjects	5th Percentile	95th Percentile
1	Weight	51.06	8.31	424	39.1	67.0
		43.37	6.55	267	34.4	56.1

To ascertain whether significant differences exist within the same population, the weight and three important dimensions viz. stature, sitting height and head circumference, were selected and their means were compared between the 7 provinces (which covered the whole country) using ANOVA. There were no significant differences in these measurements for both male and female populations, which indicated a homogeniety in body measurements among people living in one country like Sri Lanka. However the differences in means between males and females in the above measurements were highly significant ($p < 0.001$). It is believed that head and face measurements may not differ significantly between males and females in one ethnic group or society, unlike other body measurements.[9] In the Sri Lankan survey the percentage differences in mean measurements between males and females were greater in measurements taken standing (e.g. stature) (7.09%), sitting (e.g. sitting height) (7.45%) and on hand (7.13%) and on foot (7.28%) than face measurements (5.35%) as shown in Table 1. The percentage difference was least in the head measurements (3.5% approx.). Therefore with regard to the use of head gear, special modifications in sizing, seem to be not necessary between males and females.

Table 1. Percentage differences in anthropometric data between males and females

All measurements in mm.

	Stature (Stand)	Sitting Height (Sitt.)	Hand Length (Hand)	Foot Length (Foot)	Nasion–Menton (Face)	Head Length (Head)	Head Circum. (Head)
Meas. No.	17	8	88	61	77	65	63
Males	1639.01	1213.58	179.38	252.82	119.78	177.50	542.92
Females	1522.77	1123.15	166.59	234.41	113.37	171.25	523.51
Differ.	116.24	90.43	12.79	18.41	6.41	6.25	19.40
% Diff.	7.09	7.45	7.13	7.28	5.35	3.52	3.57

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3.2 Populations Compared

Most anthropometric data available today are from the United States and other European countries with emphasis on military purposes. With regard to developing countries, anthropometric data are scarce. National data are almost non-existent, as collection of such data takes a long time and effort and is sometimes not useful and feasible, considering the vastness of some countries with regard to their geographical area and climatic and ethnic differences within the country. Due to the lack of precise data for homogenous populations even in developed countries, in this paper comparisons were made from the available data which may not be statistically consistent, but suitable enough to show the variations that exist.

The following sources of data were used to make comparisons.

(a) Data provided by the national anthropometric survey of workers in Sri Lanka, (Reported in this paper).
(b) Review of literature, (Data from other countries).
(c) Results of a postal questionnaire conducted in developing countries by the authors.

In order to see whether significant variations actually exist between Sri Lankans and people from an industrially developed country e.g. Sweden, a large number of important dimensions of Sri Lankans obtained from the national survey were compared with data from Sweden.[11] Wherever data from Sweden were not available, data from Britain,[2] were substituted. It was observed that significant differences were apparent in almost all of the dimensions.

3.3 Comparison of Stature

The percentile values of stature of males of seven countries have been compared by Kennedy.[10] Values of nine other populations including Sri Lankans were included and shown in Figure 3. Making use of this graph, the percentages of accommodation of a design range of 90% of the British with respect to populations of other countries were prepared, (Table 2). It was seen that only 35% of Sri Lankans could be accommodated in such a design. Gross inadequacy of a British design (based on accommodating 90% of the British population) was seen for all countries starting from Tunisia down. Remarkably, except for Japan (an industrialised country) the problem appears to be prevalent only in developing countries.

From the available data, average statures of males and females are grouped into different regions and shown in Table 3. It can be seen from this table that North American, European and Scandinavian people are tall, African and people in the Middle East are of medium height and Latin

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Figure 3. Percentile values of statures of males from 16 countries

(IC) Industrialized Countries— (IDC) Industrially Developing Countries (Except Japan)

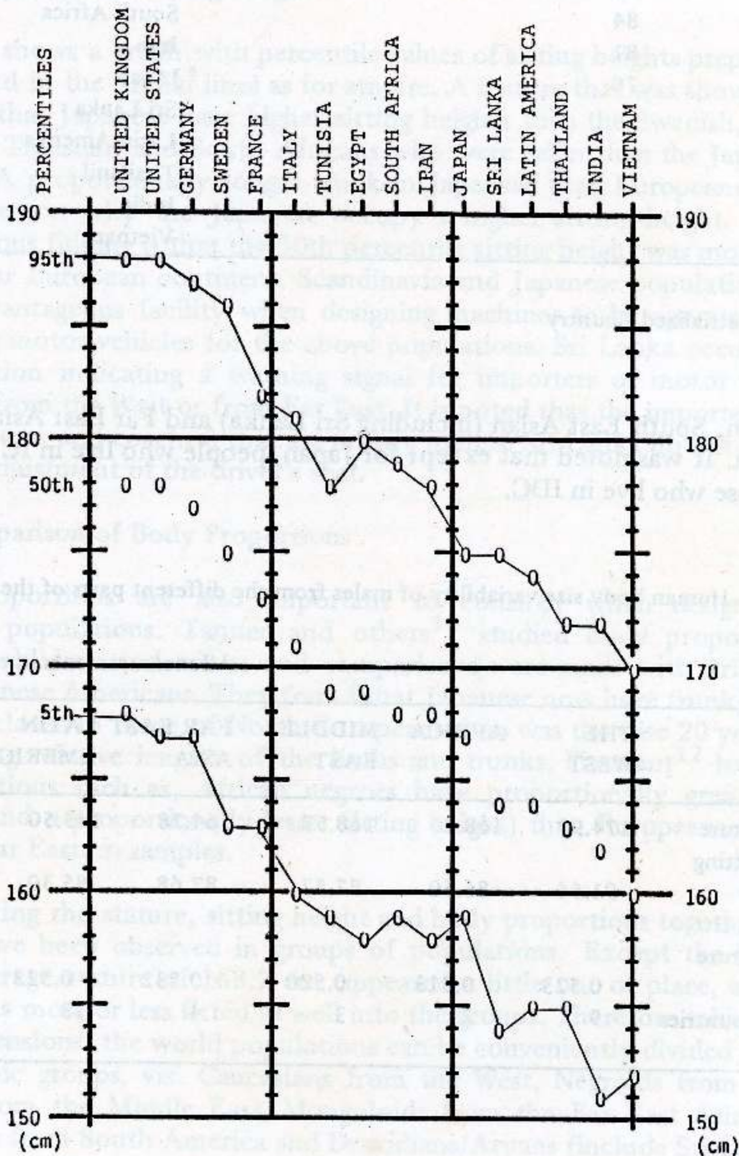

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Table 2. Percentages of accommodation of 5th to 95th percentile of a British design for other populations.

Industrialized Countries (%)		Developing Countries (%)	
United States	90	Tunisis	62
Germany	90	Egypt	59
Sweden	84	South Africa	57
France	81	Iran	52
Italy	70	* Japan	43
		Sri Lanka	35
		Latin America	32
		Thailand	24
		India	22
		Vietnam	13

* An Industrialized Country

American, South East Asian (including Sri Lanka) and Far East Asian people are short. It was noted that except for Japan, people who live in IC are taller than those who live in IDC.

Table 3. Human body size variability of males from the different parts of the world

All measurements in cm.

	THE WEST	AFRICA	MIDDLE EAST	FAR EAST ASIA	LATIN AMERICA	SOUTH ASIA
Mean Stature	174.99	168.80	168.57	164.78	163.50	163.45
Mean Sitting Height	91.54	86.60	87.57	87.68	85.30	84.25
Mean Sit. Ht/ Mean Stature Ratio	0.523	0.513	0.520	0.532	0.522	0.516
No. of Countries	9	2	3	4	18	2

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The conclusion that shorter people live in IDC may not always be true like in the example of the Japanese. Even Taiwanese from the Far East (whose data were not available at the time of writing) may be shorter people than Westerners. But Taiwan may now be classified as an IC. Therefore variability in stature and/or other dimensions can be caused by an ethnic rather than an industrial factor, although climate may also be a contributory factor, (3.5).

3.4 Comparison of Sitting Height

Figure 4 shows a graph with percentile values of sitting heights prepared and completed in the similar lines as for stature. A feature that was shown in this graph is that Japanese have higher sitting heights than the Swedish, Italians, Iranians, Tunisians and South Africans who were taller than the Japanese in stature. A proportionally longer trunk in Japanese than Europeans,[12] may be the reason why the Japanese occupy a higher sitting height. Another conspicuous finding is that the 50th percentile sitting height was more or less similar for European continent, Scandinavia and Japanese populations. This is an advantageous facility when designing machines to be operated seated including motor vehicles for the above populations. Sri Lanka occupied the last position indicating a warning signal for importers of motor vehicles, whether from the West or from Far East. It is noted that the imported motor vehicles (cars and coaches) that are mostly used in Sri Lanka do not have the vertical adjustment of the driver's seat.

3.5 Comparison of Body Proportions

Body proportions are also important to consider when designing for different populations. Tanner and others[14] studied body proportions in Japanese children and adults and comparisons were made with British and with Japanese Americans. They found that Japanese now have trunk/leg proportions closer to those of North Europeans than was the case 20 years ago. Concerning relative lengths of the limbs and trunks, Pheasant[12] had made generalisations such as, African negroes have proportionally greater limb lengths (and a proportionally lesser sitting height) than Europeans and vice versa in Far Eastern samples.

Taking the stature, sitting height and body proportions together, similarities have been observed in groups of populations. Except the Koreans where average stature of 168.7 cm appeared a little out of place, all other dimensions more or less fitted in well into the groups. Therefore taking these basic dimensions, the world populations can be conveniently divided into six main ethnic groups, viz. Caucasians from the West, Negroids from Africa, Aryans from the Middle East, Mongoloids from the Far East Asia, Latin Americans from South America and Dravidians/Aryans (include Sri Lankans) from South Asia, (Table 4).

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Figure 4. Percentile values of sitting heights of males from 15 countries (Sitting heights measured from sitting surface)

(IC) Industrialized Countries – (IDC) Industrially Developing Countries

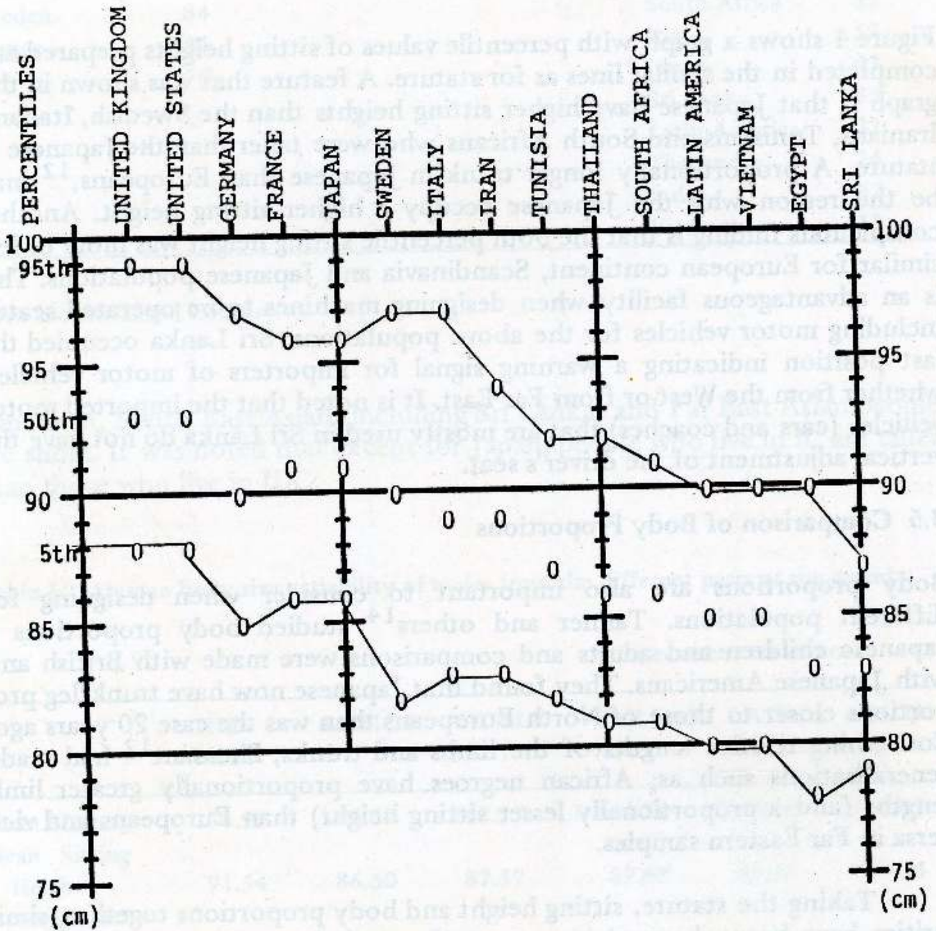

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Table 4. Average statures of males and females for groups of countries in different regions
(All measurements in centimeters)

	EUROPEAN CONTIN-ENT AND SCANDIN.	NORTH AMERICA AND CANADA	MIDDLE EAST	AFRICA	FAR EAST ASIA	LATIN AMERICA	SOUTH ASIA
MALES	174.18 (9)	177.30 (2)	168.18 (4)	168.14 (9)	165.68 (9)	165.38 (21)	164.85 (4)
FEMALES	163.80 (1)	162.00 (1)	158.00 (2)	155.92 (6)	156.49 (7)	160.00 (2)	151.43 (3)

(Number of countries appears in parenthesis)

3.6 Comparison of Hand Dimensions

In the design of machine controls, hand tools and articles where manual dexterity becomes important, the hand dimensions are crucial. Data obtained from studies conducted in the past, the hand dimensions of females from different ethnic groups and countries were compared, (Table 5). The hands of West Indian females were found significantly larger than West Europeans and Indian hands.[6] In another study, the hands of British females were found to be significantly broader than Hong Kong Chinese and Japanese hands. The Hong Kong Chinese hands were broader than Japanese hands.[4] From the other available data the hand dimensions of African, Sri Lankan, Swedish and Egyptian females were compared. Swedish hand length was significantly greater than that of Sri Lankans and Egyptians. But African (Sudanese females) hand lengths were significantly greater than Swedish hands and Sri Lankan women's hand width significantly greater than Egyptian women.

The hand dimensions do not seem to follow the same pattern as for stature and sitting heights. Women in industrialized countries had generally larger hands. The negroid women from developing countries also had larger hands, even larger than European or Caucasian women. Women from Far East Asia, Middle East and South Asia seemed to have smaller hands.

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Table 5 Mean hand measurements of females of different countries, with standard deviations and results of "t" test

All measurements in mm.

Hand Measurements	West European (W.E.) (n=51)		West Indian (W.I.) (n=20)		Indian (Panjab) (I.P.) (n=21)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Hand Length	174.3	9.3	184.0	9.6	178.5	9.2
Hand Width	92.9	5.4	97.0	5.3	94.1	5.6

Comparisons	"t" value		S/N.S		Greater Dimension	
	H. Length	H. Width	H. Length	H. Width	H. Length	H. Width
W.I. Vs. W.E.	3.77	2.85	S $p<0.0005$	S $p<0.005$	West In.	West In.
W.I. Vs. I.P.	1.83	1.66	S.$p<0.05$	N.S.	West In.	—
W.E. Vs. I.P.	1.72	0.83	S.$p<0.05$	N.S.	Indian P.	—

Hand Measurements	Hong Kong Chinese (H.K) (n=100)		British (B) (n=73)		Japanese (J) ($n>50$)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Hand Width	91.48	4.47	94.1	5.6	90.0	5.0

	"t" value	S / N.S.	Greater Dimension
H.K. Vs. B.	3.31	S. $p<0.0005$	British
H.K. Vs. J.	1.76	S. $p<0.05$	Hong Kong Ch.
B. Vs. J.	4.22	S. $p<0.0005$	British

Hand Measurements	Africa-Sudan (A.S) (n=512)		Sri Lanka (S.L) (n=287)		Sweden (S.) (n=279)		Egypt (E) (n=2200)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Hand Length	182.1	8.6	166.6	6.6	176.0	10.0	160.0	16.6
Hand Width	—		88.7	5.6	—		82.0	6.0

	"t" value		S. / N.S.		Greater Dimension	
	H.L.	H.W.	H.L.	H.W.	H.L.	H.W.
S. Vs. S.L.	13.8	—	S.$p<0.0005$	—	Swedish	—
S. Vs. E.	23.18	—	S.$p<0.0005$	—	Swedish	—
A.S. Vs. S.	8.59	—	S.$p<0.0005$	—	African	—
S.L. Vs. E.	—	18.87	—	S.$p<0.0005$	—	Sri Lanka

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3.7 Comparison of Head Dimensions

In the use of personal protective wear (ppw), viz. head gear, eye goggles, ear defenders and respirators, the head and face dimensions become important. An anthropometric survey of the head conducted by one of the authors[1] on a random sample of Sri Lankans living in England and comparison made with relevant data of the British[8] revealed significant differences even in this part of the human body. The results indicated that 12 out of 17 measures, the differences in means were significant.

Investigations on fitting of imported ppw were continued further, by matching the actual dimensions of ppw with dimensions of the users. In a pilot study conducted by one of the authors in Sri Lanka, measurements of imported ppw were made in 16 work establishments at random. Large deviations were observed when measurements were compared (Table 6).

4. Conclusions

For a successful transfer of technology or products, a knowledge of standard data of body sizes of different populations become important. Such data although available in developed countries in many forms (though may not always national data), are scarce in developing countries. Data of Sri Lankans have now been collected and compiled using simple techniques as described in this paper, which can benefit importers, manufacturers and designers of products for Sri Lankans. It is emphasized that every country should collect national data or if such data collection is not feasible, data should be collected on a geographical or district basis of homogenous populations. It is also recommended that such data be published so that they are made available and accessible to the designers and importers of goods.

The anthropometric data given in this paper are not complete and comprehensive for design, considering the miscellaneous nature of such data required for design purposes. For example when designing safety helmets, the thickness of the hair, the angle of helmet wearing, etc., become important. Therefore the data described in this paper can be treated as basic, from which design data can be developed to fit particular items of design and particular situations.

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Table 6. Results of measurements carried out on head harness of imported industrial safety helmets, goggles and hand gloves in Sri Lanka and the relevant mean anthropometric measurements of Sri Lankan and British populations (All measurements in centimeters)

1. Industrial safety helmets with adjustable head harness (3 types of helmets of a standard size)

Circumference (head harness)	Sri Lankan		British	
Percentile	5th	95th	5th	95th
Mean maximum = 66.0	51.5	57.0	55.4	59.8
Mean minimum = 54.0	(40th percentile = 54.0)			
Head length (head harness)				
Mean maximum = 24.0	15.6	19.4	18.8	20.9
Mean minimum = 16.5	(10th percentile = 16.4)			

2. Goggles (3 types)

	Sri Lankan	British
Mean interocular breadth (goggles = 2.5)	3.05	–
Mean biocular breadth (goggles = 13.25)	10.30	–

3. Industrial gloves

	Length* (gloves)	Breadth*	Hand length (mean)	Hand breadth (mean)
Large size			Sri Lanka	Sri Lanka
Sample 1	19.0	11.0		
Sample 2	19.0	12.5	(17.95)	(9.95)
Sample 3	20.0	13.0		
Small size				
Sample 4	15.0	12.0		

* The length and breadth on gloves were measured similar to measurements on human hands.

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CHEMOTAXONOMIC STUDIES OF *CROTON* SPECIES IN SRI LANKA

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Abstract : Isolation of (−)–hardwickiic acid and β–amyrin from *Croton aromaticus* and 5–hydroxy–3,7,4' –trimethoxyflavone and sitosterol from *Croton lacciferus,* and the distribution of these compounds in seven species of *Croton* are described. The contention that *C. aromaticus* and *C. lacciferus* are two distinct species is supported by the present phytochemical study.

1. Introduction

The family Euphorbiaceae consists of about 7300 species which occur mainly in the tropics and extending into the temperate regions of the northern and southern hemispheres.[11] Next to *Euphorbia,* the genus *Croton* is the largest species (700 species) of Euphorbiaceae, and many *Croton* species find application in ethnomedical preparations. Ten species of the genus *Croton* are found in Sri Lanka and no endemic species have been reported: *Croton aromaticus* Linn., *Croton bonplandianus* Haines, *Croton lacciferus* L., *Croton hirtus* L., *Croton moonii* Thw., *Croton nigroviridis* Thw., *Croton oblongifolius* Roxb., *Croton officinalis* Klotzsch, *Croton tiglium* L. and *Croton zeylanicus* Muell. As a part of our studies on medicinal plants, the chemical constituents of some *Croton* species have been examined. This report describes the isolation of (−)–hardwickiic acid and β –amyrin from *C. aromaticus,* the isolation of 5–hydroxy–3,7,4' –trimethoxyflavone and sitosterol from *C. lacciferus* and the investigation of seven species of *Croton* for the presence of these four compounds.

2. Results and Discussion

2.1 Extractives of *Croton aromaticus*

The dried roots of *C. aromaticus* were extracted with hot petroleum. The petrol extract when chromatographed over silica gel and eluted with 25%

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CH_2Cl_2–petrol gave a white crystalline solid (0.79% yield) m.p. 103–104°, $(\alpha)_D$–112.5° and a slightly less polar white solid (1.2×10^{-2}% yield), m.p. 197–198°, $(\alpha)_D$ + 87.1°. The ^1H NMR spectrum of the more polar compound (1) suggested the presence of a furan ring (δ 7.27, 7.13 and 6.18) and a carboxylic group (δ 11.57, D_2O exchangeable). The acid (1) was reacted separately with excess CH_2N_2 and excess $LiAlH_4$ to obtain the methyl ester (2) and the alcohol (3), respectively. The spectral data of 1, 2 and 3 were identical to those reported in the literature.[5,7,13] The compound (1) was thus identified as (–)–hardwickiic acid which had been isolated from *Hardwickia pinnata* (Leguminosae)[13], *Copaifera officinalis* (Leguminosae)[7] *Printzia laxa* (Compositae)[5] and *Heteropappus atlaicus* (Compositae).[6] The insecticidal properties of hardwickiic acid against *Aphis craccivora* has been reported in a recent communication.[3]

The low polar compound (4) obtained from the above column had physical data (m.p., $(\alpha)_D$, IR and ^1H NMR) consistent with those reported[9] for β–amyrin. The identity of the compound (4) as β–amyrin was confirmed by direct comparison with an authentic sample.

2.2 Extractives of *Croton lacciferus*

Crushed fresh leaves of *C. lacciferus* were extracted with methanol under reflux conditions. The hot methanol extract when chromatographed over silica gel and eluted with 2% EtOAc–benzene gave a yellow crystalline solid (5) m.p. 147–148° and a white crystalline solid (6) m.p. 136–137°, $(\alpha)_D$–36°. The UV λ_{max} of the yellow compound (5) at 345 nm and positive Gibbs test indicated the presence of a flavone system containing a phenolic group with the *para* position unsubstituted. ^1H NMR spectrum suggested the presence of three methoxy groups (δ 3.89 and 3.87), two aromatic hydrogens in a *meta* relationship (δ 6.42, 6.32, J = 2Hz) and four hydrogens of a *para* substituted benzene ring (δ 8.07, 6.49, J = 9Hz). These data and the molecular formula $C_{18}H_{16}O_6$ (high resolution mass) are consistent with those for a trimethoxy derivative of keampferol. The structure of this compound was established as 5–hydroxy–3,7,4' –trimethoxy flavone (5) by comparison of its physical data (m.p., $(\alpha)_D$, IR, ^1H NMR and mass) with those reported for the flavone (5) which had been isolated from the fern *Chelianthes farinosa* (Gymnogrammoideae).[8]

The compound (6) obtained from the leaf extract of *C. lacciferus* was found to be sitosterol by direct comparison of its physical data[9] with those of an authentic sample.

2.3 Phytochemical screening of *Croton* species

The presence of (–)–hardwickiic acid (1),β–amyrin (4) and sitosterol (6) has

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been reported in some *Croton* species previously: (1) in *C. oblongifolius*[2] and *C. californicus*[12], (4) in *C. caudatus*[14] and (6) in *C. caudatus*[14] and *C. oblongifolius.*[15] These results together with the present findings from the investigation of *C. aromaticus* and *C. lacciferus* prompted us to screen locally available *Croton* plants for the presence of compounds (1), (4), (5) and (6).

Leaves and roots of seven *Croton* species were collected from different parts of Sri Lanka. The dried leaves and roots were separately extracted with hot chloroform. The TLC's of these extracts were compared with those of the corresponding extracts from *C. aromaticus* and *C. lacciferus* and the authentic samples of (−)–hardwickiic acid (1) and the trimethoxyflavone (5).

A greyish blue spot with a conical shape, corresponding to (−)–hardwickiic acid (1) was observed on TLC ($CHCl_3$, anisaldehyde) in the root extracts of *C. aromaticus* only. The trimethoxyflavone (5) was observed in the leaf extracts of *C. lacciferus* and *C. oblongifolius* as a yellow spot on TLC (5% EtOAc–benzene, anisaldehyde). Whether or not β–amyrin (4) and sitosterol (6) were present in the extracts could not be determined using the TLC method as the corresponding spots were obscured and/or inconspicuous. Hence an HPLC analysis of the extracts was carried out.

Chloroform solutions of the pure compounds, extracts, and extracts mixed with the four compounds were separately injected to an HPLC instrument with attachment to an RI detector, and the chromatograms recorded. The detection of the compounds in each extract was based on their retention times and enhancement of peaks in the chromatogram of the sample containing the extract and four compounds. The results are given in Table 1.

In conformity with the results of the TLC studies, the HPLC analysis also revealed the presence of (−)–hardwickiic acid (1) in *C. aromaticus* (roots) and the flavone (5) in *C. lacciferus* (leaves) and *C. oblongifolius* (leaves). However, the HPLC results further suggested that trace amounts of (−)–hardwickiic acid (1) were present in *C. lacciferus* (roots), *C. oblongifolius* (leaves) and *C. officinalis* (roots). Similarly the presence of the flavone (5) in the leaf and the root extracts of *C. tiglium* was detected only in the HPLC study. While all the plants except *C. oblongifolius* contained sitosterol (6), β–amyrin (4) was found in *C. aromaticus, C. bondplandianus, C. lacciferus* and *C. officinalis.*

2.4 Chemotaxonomic significance

Trimen[16] regarded *C. aromaticus* as a variety of *C. lacciferus* although other botanists[1,10] consider the two taxa as distinct species. In a previous study,[4] it has been found that the roots of *C. lacciferus* contained several kauranoids while these compounds were absent in the roots of *C. aromaticus.* Together with the results of the present study (Table 1), it is clear that the nature and

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Table 1. Distribution[a] of (–)–hardwickiic acid (1), β–amyrin (4), 5–hydroxy–3,7,4'–trimethoxy–flavone (5) and sitosterol (6) in *Croton* species

Plant	Site of collection	Plant part[b]	1	4	5	6
Croton aromaticus	Nakkala	LF	–	–	–	+[c]
	Kithulhitiyawa	RT	++[c]	++	–	–
Croton bonplandianus	Madduvil	LF	–	++	–	++
	Kalpitiya	RT	–	–	–	++
Croton hirtus	Nakkala	LF	–	–	–	++
	Mihintale	RT	–	–	–	++
Croton lacciferus	Dawulagala	LF	–	+	++	++
		RT	+	–	–	+
Croton oblongifolius	Maha Oya	LF	+	–	++	–
Croton officinalis	Peradeniya	LF	–	+	–	+
	Nochchiyagama	RT	+	++	–	–
Croton tiglium	Peradeniya	LF	–	–	++	++
		RT	–	–	++	++

[a]From HPLC analysis

[b]LF = leaves, RT = roots

[c] + = less than 0.6% in the extract, ++ = more than 0.6% in the extract, as calculated from the HPLC chromatograms.

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the distribution of chemical constituents in *C. aromaticus* and *C. lacciferus* are different. The view that these two taxa be maintained as two distinct species is thus supported by the phytochemical studies.

3. Experimental

3.1 Investigation of *Croton aromaticus*

Dried roots (4.3 kg) of *C. aromaticus* collected at Kithulhitiyawa near Dambulla, were crushed and extracted exhaustively with petroleum (bp 60–80°C) under reflux conditions for 5 days. The removal of solvent on a rotavapor gave a brown semi-solid (110 g).

3.1.1 Isolation of β–amyrin (4)

Flash chromatography of the petroleum extract over silica gel (TLC grade, Merck) with 25% CH_2Cl_2–petroleum afforded a white crystalline solid mp 197–198°C (CH_2Cl_2–petroleum) (lit.[9] 197–197.5°C); $(\alpha)_D$ + 87.1° ($CHCl_3$, c, 0.98) (lit.[9] 88.4°). This compound was found to be identical with an authentic sample of β–amyrin (mp, mmp, Co–TLC, IR and ^1H NMR).

3.1.2 Isolation of (–)–hardwickiic acid (1)

Further elution of the above column with 25% CH_2Cl_2–petroleum followed by TLC ($CHCl_3$) furnished a white crystalline solid (3.09 g), mp 103–104°C (n–hexane), (lit.[13] 106–107°C); $(\alpha)_D$–112.5°($CHCl_3$, c, 0.48) (lit.[13] 114.7°). This compound was identified as $(\alpha)_D$–hardwickiic acid from the following spectroscopic data and chemical conversions; IR ν_{max}(KBr) 3340–2200(OH), 1670(CO), 1400, 1255, 1240 and 775 cm^{-1}; ^1H NMR δ ($CDCl_3$, 60 MHz) 11.57 (1H,brs, D_2O exchangeable, CO_2H), 7.27 (1H, t, J = 2 Hz, 16–H), 7.13(1H,brs, 15–H), 6.83 (1H, t, J = 4Hz, 3–H), 6.18 (1H, brs, 14–H), 2.70–1.20 (15H, m), 1.23 (3H, s, 18–H), 0.83(3H, d, J = 8Hz, 17–H), 0.77(3H, s, 20–H); MS m/z (rel. int.) 316(M^+, 43), 301(20), 283(100), 221(40), 203(34), 137(22), 125(75), 96(37), 81(59).

3.1.3 Methylation of (–)–hardwickiic acid (1) into ester (2)

(–)–Hardwickiic acid (1) (27 mg) was dissolved in Et_2O (1 ml) and treated with excess ethereal CH_2N_2 for 30 min. After removal of the solvent, the product was purified by TLC (20% CH_2Cl_2–petroleum) to give the ester (2) as a colourless oil (26 mg, 92%), $(\alpha)_D$–95.1° ($CHCl_3$, c, 1.02); IR ν_{max} (neat) 1710(CO), 1430, 1245 and 1230 cm^{-1}; ^1H NMR δ(CCl_4)

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7.30 (1H, t, J = 2 Hz, 16–H), 7.13 (1H, brs, 15–H), 6.57 (1H, t, J = 4 Hz, H–3), 6.17 (1H, brs, 14–H), 3.62 (3H, s, OMe), 2.70–1.20 (15H, m), 1.23 (3H, s, 18–H), 0.84 (3H, d, J = 8 Hz, 17–H), 0.77 (3H, s, 20–H).

3.1.4 Reduction of (–)–hardwickiic acid (1) into alcohol (3)

$LiALH_4$ (25 mg) was added portion-wise to a solution of 1 (20 mg) in Et_2O and stirred at room temperature for 12h. Usual work up gave a residue which was purified using TLC (50% CH_2Cl_2–petroleum) to give the alcohol (3) as a colourless oil (19 mg, 80%), $(\alpha)_D$ –34.5° ($CHCl_3$, c, 0.58); IR ν_{max} (neat) 3600–3050(OH), 1450, 1380 and 1020 cm^{-1}; ^1H NMR δ (CCl_4) 7.27 (1H, t, J = 2Hz, 16–H), 7.13 (1H, brs, 15–H), 6.17 (1H, brs, 14–H), 5.50 (1H, t, J = 4 Hz, 3–H), 3.93 (2H, brs, 19–H), 2.70–1.20 (15H, m), 1.07 (3H, s, 18–H), 0.81 (3H, d, J = 8Hz, 17–H), 0.75 (3H, s, 20–H).

3.2 Investigation of *Croton lacciferus*

Fresh leaves (250 g) of *C. lacciferus* collected at Dawulagala near Peradeniya, were crushed and exhaustively extracted with MeOH under reflux conditions for 5 days. The removal of MeOH on a rotavapor gave a semi-solid (29 g).

3.2.1 Isolation of 5–hydroxy–3,7,4'–trimethoxyflavone (5)

Silica gel column chromatography (2% EtOAc–C_6H_6) of the MeOH extract (20 g) followed by TLC (5% EtOAc–C_6H_6) gave a yellow crystalline solid (142 mg) which was identified as the flavone (5), mp 147–148°C ($CHCl_3$–MeOH)(lit.[8] 144–147°C); UV λ^{MeOH}_{max} (log ϵ)345(4.34), 268(4.40), 210(4.50) nm; IR ν_{max}(KBr) 1655(CO), 1585, 1490, 1460 cm^{-1}; ^1H NMR δ ($CDCL_3$) 12.60 (1H, s, 5–OH), 8.07, 6.49 (2H each, d, J = 9Hz, 2'–H, 3'–H, 5'–H, 6'–H), 6.42, 6.32 (1H each, d, J = 2Hz, 6–H, 8–H), 3.89 (3H, s, OMe), 3.87 (6H, s, 2xOMe); MS m/z (rel. int.) 328 (M+, 100), 327 (94), 285 (63), 242 (18), 150 (15), 148 (150, 135 (20), 119 (12); $C_{18}H_{16}O_6$ (M+) 328.0957 (cal. 328.3246).

3.2.2 Isolation of sitosterol (6)

Further elution of the above column with 2% EtOAc–C_6H_6 gave a white crystalline solid (56 mg), mp 136–137° C (lit.[9] 136–137°C); (α)D–36° ($CHCl_3$, c, 1.30) (lit.[9] –35°). This compound was found to be identical to an authentic sample of sitosterol (mp, mmp, Co–TLC, Ir and ^1H NMR).

3.3 Phytochemical screening

Leaves and roots of *C. aromaticus, C. bonplandianus, C. hirtus, C. lacciferus, C. oblongifolius, C.officinalis* and *C. tiglium* were collected from different

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parts of Sri Lanka (Table 1). The air-dried leaves and roots were separately extracted with chloroform under reflux conditions for 24 h. The residues produced from concentrating the extracts were used in the following TLC and HPLC analyses.

3.4.1 TLC analysis

Each chloroform extract of the above plants, the petroleum extract of the roots of *C. aromaticus* and (–)–hardwickiic acid (1) were spotted on TLC plates (silica gel), and the plates were developed in $CHCl_3$, dried in air and sprayed with anisaldehyde. A greyish blue spot with a conical shape corresponding to (–)–hardwickiic acid (1), was observed only in the root extracts of *C. aromaticus.*

Each chloroform extract of the *Croton* plants, the MeOH extract of the leaves of *C. lacciferus* and the flavone (5) were spotted on TLC plates. A yellow spot (5% EtOAc–C_6H_6, anisaldehyde spray) corresponding to the flavone (5) was observed in the leaf extracts of *C. lacciferus* and *C. oblongifolius.*

3.4.2 HPLC analysis

The presence of (–)–hardwickiic acid (1), β–amyrin (4), flavone (5) and sitosterol (6) in the $CHCl_3$ extracts of the *Croton* species was studied using a high performance liquid chromatography (HPLC) instrument equipped with Waters Associates chromatography pump, a Differential Refractometer (R 401, Waters) and a normal phase column (450 x 10 nm, Partisil M9 10/15) connected to a guard column (CO–PELL ODS, Whatman). Eighty percent $CHCl_3$–n–hexane was used as the mobile phase maintaining a flow rate of 2.5 ml/min. Retention time of each compound was determined by injecting a $CHCl_3$ solution of each compound (0.05 mg, in 25 μl); the retention times of 1, 4, 5 and 6 were found to be 22.0 17.6, 13.2 and 24.0 min, respectively. $CHCl_3$ solutions of each extract (0.04 mg/μl) and the mixture of pure compounds (0.002 mg/μl for each compound) were prepared for subsequent injections. For each extract two HPLC runs were made by separately injecting the $CHCl_3$ solution of the extract (100 μl) and another solution prepared by mixing the $CHCl_3$ solutions of the extract (100 μl) and another solution prepared by mixing the $CHCl_3$ solutions of the extract (100μl) and the compound mixture (25 μl). The presence of the compounds in each extract signified by their retention times, was confirmed by observing the peak enhancements in the chromatogram corresponding to the combined solution of the extract and the mixture of four compounds. The results are given in Table 1.

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LUDWIGIA DECURRENS WALT. — A NEW RICE–FIELD WEED IN SRI LANKA

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(Date of receipt : 26 May 1986)
(Date of acceptance : 22 December 1987)

Abstract : *Ludwigia decurrens,* a member of the family Onagraceae, is a new weed in the rice-fields of Sri Lanka. It has probably been introduced to the country relatively recently. This weed is presently found in great abundance in the rice-field habitats of Colombo and Kalutara districts, and to a lesser extent in Gampaha, Kegalle, Ratnapura and Galle districts. The weed produces large numbers of seeds which are easily spread by irrigation water and rain water. Successful management of the spread of this weed will depend on applying control measures before the plant comes into bloom and sets seeds. Its botanical features are described.

1. Introduction

The genus *Ludwigia* belongs to the family Onagraceae, a family that has many temperate and sub-tropical species which are mainly herbs, but includes some shrubs as well. The family Onagraceae also includes the genus *Jussiaea* well-known to botanists. However, due to evidence of many close relationships and inter-connections between *Jussiaea* and *Ludwigia*, the two genera have now been merged and the name *Ludwigia* accepted as the generic name most tenable for the aggregate genus.[4]

Trimen[5] in the Handbook to the Flora of Ceylon (1893–1900) described two species of *Ludwigia,* namely *Ludwigia parviflora* Roxb. and *Ludwigia prostrata* Roxb., as well as two species of *Jussiaea,* namely *Jussiaea repens* L. and *Jussiaea suffruticosa* L. as occurring in Sri Lanka. He also noted that a variety of *J. suffruticosa* L. var. *β subglabra* Thw., appeared so different from the type *(J. suffruticosa)* and stated that he was inclined to make a second species. Trimen also stated that L. *prostrata* was very rare and that he had only seen the specimen collected by Moon at Kalutara.

Alston[1] in his Supplement to Trimen's Flora of Ceylon, viz. Vol VI (1931) recorded four species of *Jussiaea* for Sri Lanka. He accepted the previously described *J. repens* L., but did several alterations to the other species recorded by Trimen. The name of *J. suffruticosa* sensu Trimen was altered to *Jussiaea peruviana* L., while the name *J. suffruticosa* L. was retained for the variety *subglabra* noted earlier. Alston also described a new

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species, *Jussicea tenella* Burm. f. Of the *Ludwigia* species, Alston altered the name of *L. parviflora* sensu Trimen, to read as *Ludwigia perennis* L.

Chandrasena and Amarasinghe[3] in a preliminary communication, presented evidence of seven species of *Ludwigia* found growing naturally in Sri Lanka. They also favoured the view that the name *Ludwigia* be accepted as the valid name in describing these species, following Raven. The seven species described were: *Ludwigia adscendens* (L.) Hara *(=J. repens), Ludwigia peruviana* (L.) Hara (=J. *peruviana), Ludwigia octovalvis* (Jacq.) Raven *(=J. suffruticosa), L. perennis, Ludwigia hyssopifolia* (G. Don) Exell *(=J. tenella), Ludwigia uruguayensis* (Camb.) Hara and *Ludwigia decurrens* Walt. They stated that *L. prostrata,* which Trimen himself had not collected, was never found by them despite extensive searching and preferred to leave this species out. Their list included *Ludwigia uruguayensis* and *L. decurrens,* two species which had not been previously recorded from Sri Lanka. Whilst *L. uruguayensis* was of rare occurrence, *L. decurrens* was reported as a new rice-field weed of considerable importance, already well established in the Wet Zone.[3]

The objective of the present paper is to record the occurrence, botanical nature, present distribution and significance of *L. decurrens,* which is a new species for Sri Lanka.

2. Botanical Characteristics of *Ludwigia decurrens*

Ludwigia decurrens Walt. Fl. Carolin. 89. 1788; Raven, Reinwardtia 6:347. 1963.

= *Jussiaea decurrens* (Walt.) Dc., Prod. 3: 56. 1828; Munz, Darwiniana 4: 198; Brenan in Hutch. & Dalz., Fl. W. Trop. Afr. ed. 2, 1:169. 1954.

A small erect herb up to 1.5 m tall, well-branched, woody when mature, stem glabrous, 4-winged from the decurrent leaf bases and the wings 1–2 mm wide. Leaves alternate, glabrous, sessile or sub-sessile, narrowly lanceolate to elliptical in shape, 2–12 cm long, 0.2–3 cm broad at broadest point, acute to acuminate at apex, cuneate at base, venation reticulate with 12–15 main veins on each side of mid-rib, sub-marginal vein prominent (Figure 1). Flowers borne singly in upper leaf axils, sessile; Sepals 4, lance-ovate, 7–10 mm long, 2–3 mm broad, glabrous; Petals 4, bright yellow, broadly ovate, 8–14 mm long, 6–10 mm broad at broadest point; Stamens 8, in two whorls, epipetalous whorl slightly shorter; filaments 1–3 mm long, anthers 1–2 mm long with sub-versatile attachment. Pollen shed in tetrads. Style 2–3 mm long, stigma globose, green 1–2 mm thick. Ovary inferior, 1–1.2

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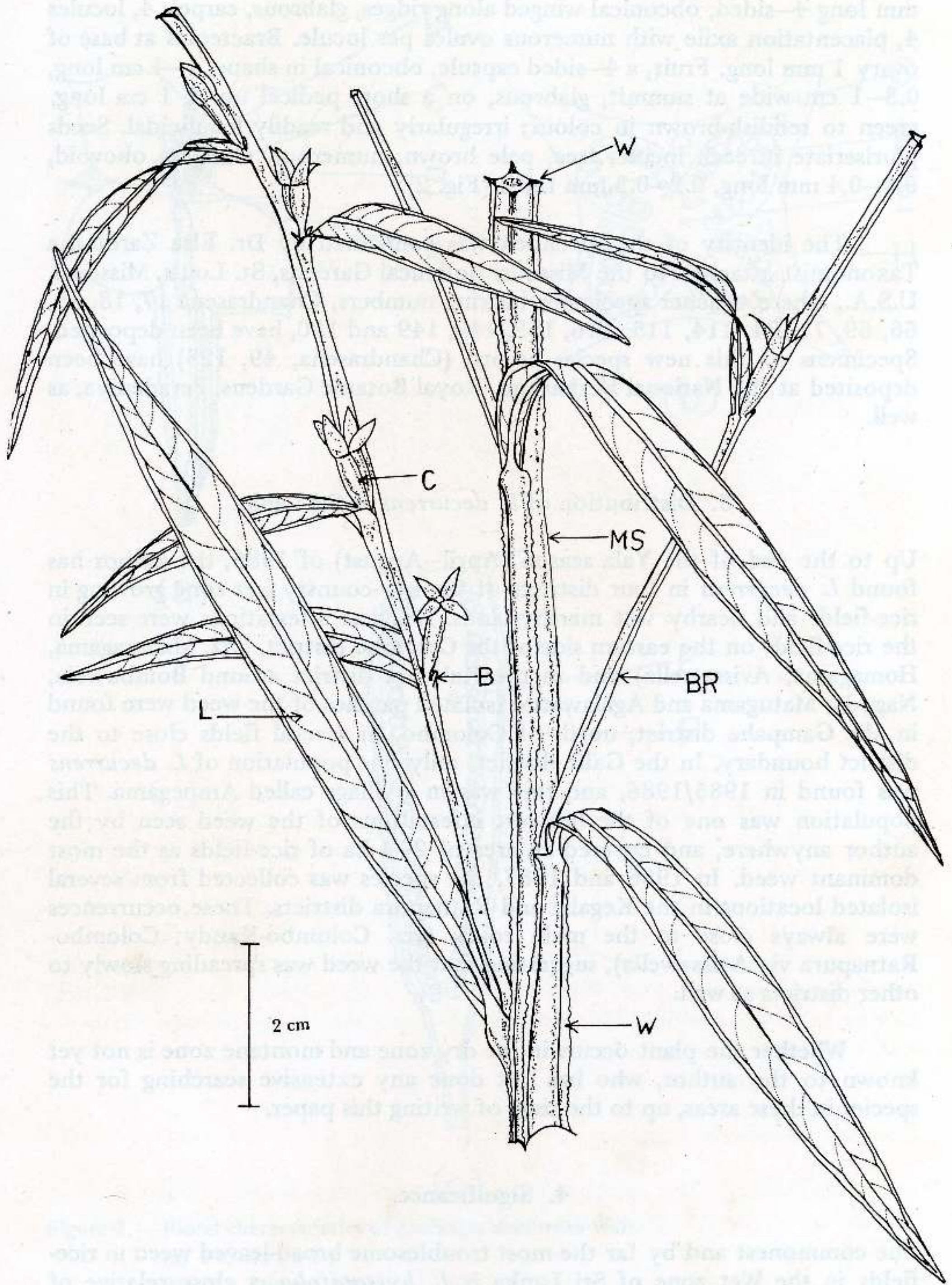

Figure 1. Habit of *Ludwigia decurrens* Walt. (x 1). main stem (MS), branch (BR), leaf showing reticulate venation and sub-marginal vein (L), bracteoles at base of ovary/capsule (B), capsule (C), wings from decurrent leaf bases (W).

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mm long 4–sided, obconical winged along ridges, glabrous, carpels 4, locules 4, placentation axile with numerous ovules per locule. Bracteoles at base of ovary 1 mm long. Fruit, a 4–sided capsule, obconical in shape, 1–4 cm long, 0.3–1 cm wide at summit, glabrous, on a short pedicel up to 1 cm long, green to reddish-brown in colour; irregularly and readily loculicidal. Seeds pluriseriate in each locule, free, pale brown, numerous, elongate obovoid, 0.3–0.4 mm long, 0.2–0.3 mm thick (Fig. 2).

The identity of the specimen was confirmed by Dr. Elsa Zardini, a Taxonomist attached to the Missouri Botanical Gardens, St. Louis, Missouri, U.S.A., where voucher specimens bearing numbers, Chandrasena 17, 18, 65, 66, 69, 71, 74, 114, 115, 116, 119, 146, 149 and 150, have been deposited. Specimens of this new species record (Chandrasena, 49, 123) have been deposited at the National Herbarium, Royal Botanic Gardens, Peradeniya, as well.

3. Distribution of *L. decurrens* in Sri Lanka

Up to the end of the Yala season (April–August) of 1986, the author has found *L. decurrens* in four districts of the low-country wet zone growing in rice-fields and nearby wet marshy lands. Heaviest infestations were seen in the rice-fields on the eastern side of the Colombo district, (viz. Maharagama, Homagama, Avissawella) and in the Kalutara district around Bombuwela, Nagoda, Matugama and Agalawatta. Isolated patches of the weed were found in the Gampaha district, north of Colombo, in several fields close to the district boundary. In the Galle district, only one population of *L. decurrens* was found in 1985/1986, and this was in a village called Ampegama. This population was one of the heaviest infestations of the weed seen by the author anywhere, and covered an area of 2–4 ha of rice-fields as the most dominant weed. In 1986 and 1987, the species was collected from several isolated locations in the Kegalle and Ratnapura districts. These occurrences were always close to the main roads (viz. Colombo-Kandy; Colombo-Ratnapura via Avissawella), suggesting that the weed was spreading slowly to other districts as well.

Whether the plant occurs in the dry zone and montane zone is not yet known to the author, who has not done any extensive searching for the species in these areas, up to the time of writing this paper.

4. Significance

The commonest and by far the most troublesome broad-leaved weed in rice-fields in the Wet zone of Sri Lanka is *L. hyssopifolia,* a close relative of

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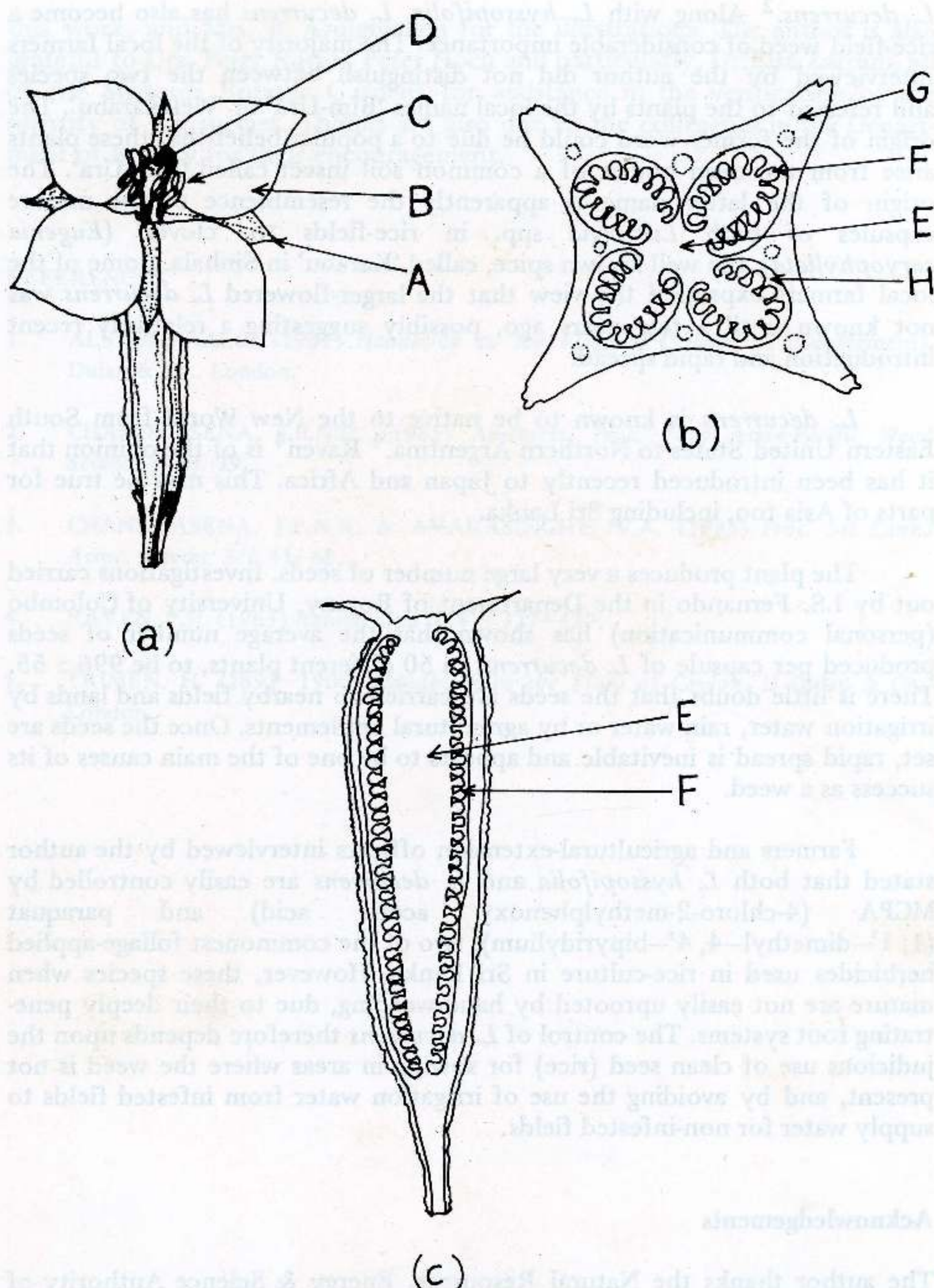

Figure 2. Floral characteristics of *Ludwigia decurrens* Walt.
(a) Flower (x 2)
(b) T.S. Ovary (x 4)
(c) L.S. Ovary (x 4)
Sepals–4 (A), Petals–4 (B), Stamens–8 (C), Stigma (D), axis of ovary (E), Ovules– numerous (F), Carpels–4 (G), Locules–4 (H).

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L. decurrens.[2] Along with *L. hyssopifolia, L. decurrens* has also become a rice-field weed of considerable importance. The majority of the local farmers interviewed by the author did not distinguish between the two species and referred to the plants by the local names 'Bim-Uru' or 'Wel-Karabu'. The origin of the former word could be due to a popular belief that these plants arise from the dead bodies of a common soil insect called 'Bim-Ura'. The origin of the latter name is apparently the resemblence of the mature capsules of both *Ludwigia* spp. in rice-fields to 'cloves' (*Eugenia caryophyllata*), the well known spice, called 'Karabu' in Sinhala. Some of the local farmers expressed the view that the larger-flowered *L. decurrens* was not known until a few years ago, possibly suggesting a relatively recent introduction and rapid spread.

L. decurrens is known to be native to the New World from South Eastern United States to Northern Argentina.[4] Raven[4] is of the opinion that it has been introduced recently to Japan and Africa. This may be true for parts of Asia too, including Sri Lanka.

The plant produces a very large number of seeds. Investigations carried out by I.S. Fernando in the Department of Botany, University of Colombo (personal communication) has shown that the average number of seeds produced per capsule of *L. decurrens,* in 50 different plants, to be 996 ± 55. There is little doubt that the seeds are carried to nearby fields and lands by irrigation water, rain water or by agricultural implements. Once the seeds are set, rapid spread is inevitable and appears to be one of the main causes of its success as a weed.

Farmers and agricultural-extension officers interviewed by the author stated that both *L. hyssopifolia* and *L. decurrens* are easily controlled by MCPA (4-chloro-2-methylphenoxy acetic acid) and paraquat (1, 1'–dimethyl–4, 4'–bipyridylium), two of the commonest foliage-applied herbicides used in rice-culture in Sri Lanka. However, these species when mature are not easily uprooted by hand-weeding, due to their deeply penetrating root systems. The control of *L. decurrens* therefore depends upon the judicious use of clean seed (rice) for sowing in areas where the weed is not present, and by avoiding the use of irrigation water from infested fields to supply water for non-infested fields.

Acknowledgements

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this work, and Mrs. P. Arulgnanam for the illustrations. The author is also grateful to Drs. Peter Raven, Peter Hoch and particularly, Dr.Elsa Zardini, all of the Missouri Botanic Gardens for assistance in the verification of the identity of the species. Thanks are also due to the colleagues in the Department of Botany for their encouragement.

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IRON CONTAMINATION DURING COMMERCIAL GRINDING OF SPICES

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Abstract : The concentration of copper, iron and zinc were estimated by atomic absorption spectrophotometry in powdered spices purchased from retailers. The three elements were compared by grinding the whole spices by mortar and pestle, grinding stone, food blender and in commercial mills. The iron, due to wear and tear of the machinery, contaminate the spices during commercial grinding increasing the iron content 3–5 fold; the concentrations being as high as 750 μg/g of ground spices. Highest contamination were seen in turmeric powder. The iron contaminated spices are ferromagnetic confirming the particulate nature of iron in spices. The copper and zinc content were comparable in the replicates ground by different methods as well as in the samples purchased from retailers. Further study on the nutritional significance of iron contamination in ground spices appears warranted.

1. Introduction

Spices are added to enhance the flavour, aroma and colour of food but are not considered as sources of minerals or other nutrients. Ground spices are reported to contain high iron concentrations, sometimes 3–4 times higher than in other edible plant components, implicating possible nutritional benefits through the spices.[3,6] However, the origin of such high levels of iron is not understood.

In this study, experiments were directed at determining the origin of the copper, iron and zinc content of ground spices.

2. Materials and Methods

2.1 Spices

The following whole or commercially ground spices and curry powder were purchased from the retailers in Gampaha, Kandy, Matale, Nugegoda, Peradeniya and Warakapola.

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Pods of ripe chillie *(Capsicum annuum)*
Berries of pepper *(Piper nigrum)*
Rhizomes of turmeric *(Curcuma longa)*
Seeds of coriander (*Coriandrum sativum)*
Fruits of cumin (*Cuminum cyminum)*

2.2 Grinding

Whole spices were washed with demineralized water, and dried at 70°C in the vacuum oven. Replicate samples of the dried spices were ground by the following methods:

(a) In a laboratory mortar and pestle
(b) In a clean grinding stone at kitchen
(c) In a food blender (National Model MX–11PN)
(d) In commercial mills in Kandy

Estimations were made from triplicate samples ground by each method.

2.3 Estimation of copper, iron and zinc

The powdered spices (1g) were ignited in a muffle furnace at 460°C for eight hours. This was moistened with glass distilled demineralized water (2 ml) and concentrated nitric acid (5 ml) and warmed for few minutes over the flame. The solutions were filtered in to 100 ml volumetric flasks through Whatman No. 541 filter paper and made up to the mark.

The estimations were carried out using a Perkin–Elmer Model 2380 Atomic Absorption Spectrophotometer containing an adjustable titanium burner head and air/acetylene flame, using high intensity "Intensitron" lamps at the wave lengths and slit widths given in Table 1.[7]

Table 1

element	wave length (nm)	slit (nm)
copper	324.8	0.7
iron	248.3	0.2
zinc	213.9	0.7

The quantitative analysis of the elements were conducted using standard curves obtained using the same instrument, for standard solutions containing the respective cations.[2] The coefficient of variation for the determinations varied up to 15% for the home ground samples and up to 40% for

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the mill ground samples; the values for commercially purchased samples varied between 15–79%.

2.4 Ferromagnetism

The powdered spices were firmly packed to a length of 4 cm in a glass tube of 10 cm x 10 mm (internal diameter) and suspended in a double pan laboratory balance at the centre of an electromagnet set underneath the balance. The tube was balanced using the weights.

A magnetic field strength of up to 3000 oersted was produced by the electromagnet with pole gap of 2 cm, resistance of 15.3 ohm and 2337 turns which was supplied with a power of 22 volts and 4.5 amp to produce a magnetic flux of 60 maxwell turns using Eleminac type B 2027 generator. The magnetic flux produced was measured by a calibrated fluxmeter in maxwell turns per 100 cm^2 search coil.[5]

3. Results and Discussion

3.1 Commercially ground spices

The powdered spices purchased from the retailers contained high iron content as already reported [6] and the copper and zinc contents were much less than iron (Table 2). The iron content varied widely among the samples with standard deviations up to 70% of the mean whereas the standard deviations for copper and zinc contents were less than 30% of the mean. The high inhomogeneity of iron content in commercial spice powders suggest the presence of iron as a contaminant rather than a naturally occurring constituent of the raw materials.

The iron content in the commercial powders did not show any correlation with the location of purchase. Of the different types of spices examined turmeric had the highest iron content (Table 2).

3.2 Home ground spices

The spices ground by three methods at home/laboratory showed comparable figures for copper, iron and zinc content (Table 3). The iron content found in the home ground spices was only 25 to 35 per cent of the levels reported in commercially ground spices. In contrast to the iron content, the copper and zinc contents were the same in both home ground and commercially purchased spices further indicating the high iron content to be a contamination during grinding in commercial mills.

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Table 2. Copper, iron and zinc concentrations (μg/g) in commercial powdered spices purchased from retailers.

Spice / element	No. of samples	Mean	S.D.	Range
Chillie	25			
copper		36.3	14.4	22–64
iron		458.0	144.2	214–806
zinc		20.0	7.3	10–30
Curry	16			
copper		33.1	5.8	22–41
iron		608.1	396.1	197–1998
zinc		29.0	4.4	24–40
Pepper	5			
copper		52.8	11.8	38–71
iron		449.4	326.0	100–912
zinc		19.4	8.4	8–28
Turmeric	15			
copper		17.3	13.7	1–47
iron		740.6	491.4	107–1617
zinc		27.1	12.7	6–60

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Table 3. Copper, iron and zinc concentrations ($\mu g/g$) in spices ground using mortar and pestle, grinding stone or food blender.

Spice	element	Method of grinding			Mean ± S.D.
		mortar & pestel	grinding stone	food blender	
Chillie (3)					
	copper	49	43	41	44.3 ± 3.3
	iron	126	138	133	132.3 ± 4.9
	zinc	18	16	12	15.3 ± 2.4
Coriander (3)					
	copper	41	38	35	38.3 ± 2.4
	iron	143	146	147	145.3 ± 1.6
	zinc	32	30	28	30.0 ± 1.6
Cumin (3)					
	copper	49	49	44	47.3 ± 2.3
	iron	170	178	167	171.6 ± 4.6
	zinc	30	28	25	27.6 ± 2.0
Pepper (3)					
	copper	54	52	54	53.3 ± 0.9
	iron	74	70	68	70.6 ± 2.4
	zinc	24	25	28	25.6 ± 1.6
Turmeric (3)					
	copper	30	25	24	26.3 ± 2.6
	iron	130	143	140	137.6 ± 5.5
	zinc	28	28	29	28.3 ± 0.4

Results for the same samples ground in the mill.

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Among the spices cumin seed is reported to be a naturally rich source of iron[4] containing 950 μg/g. The samples examined in this study contained only 170 μg/g of iron in cumin seeds.

3.3 Commercial grinders

When replicates from the whole spices ground at home were ground in commercial grinders, a 3–5 fold increase in the iron content was noted whereas the copper and zinc contents were the same as in home ground spices (Figure 1) confirming that iron contamination occurs during grinding. The added iron content varied from mill to mill widely. Of the 10 grinding mills compared for added iron content (Table 4) the mill No. 1 exhibited highest contamination for all types of spices.

The grinders used in Sri Lanka are of "burr type" and consist of two roughened cast iron discs, where flutes are grooved in the two facing sides. One plate rotates on a shaft and the whole spices are fed between the plates for grinding by crushing and shearing action. It is quite possible that iron, in a fine particulate form, is produced due to wear and tear during grinding. The higher iron content noted with turmeric (Table 2 and 4) is probably due to higher hardness of turmeric rhizomes causing greater wear and tear compared with the other spices. It is also possible that higher iron contamination occur in grinding mills where the plates are new and rough.

3.4 Magnetic properties

If iron contamination occurs in spices during grinding, the particulate nature of iron should add a ferromagnetic effect to the ground spices when tested by Gouy method.[5] Home ground spices when subjected to a magnetic field were diamagnetic, exerting no force whereas the commercially ground spices exhibited a positive force on the balance confirming the particulate nature of iron. However, the ferromagnetic force was not sufficiently strong to be measured under our experimental conditions.

3.5 Nutritional significance

In cooking foods the total amount of spices added average to 5–10 g per person with a possible daily contribution of 5 mg iron per person from spices containing about 500 μg/g of iron, as observed in this study. This falls very close to the recommended daily dietary allowance of 6–15 mg or iron.[8] However, iron existing in the ferric state in the powdered spices may not be readily available for absorption during digestion.[10] The bioavailability of iron depends on several parameters. On one hand the presence of ascorbic acid and citric acid contributed from lime during cooking of vegetables may convert the iron into chelate forms that could be readily absorbed. On the

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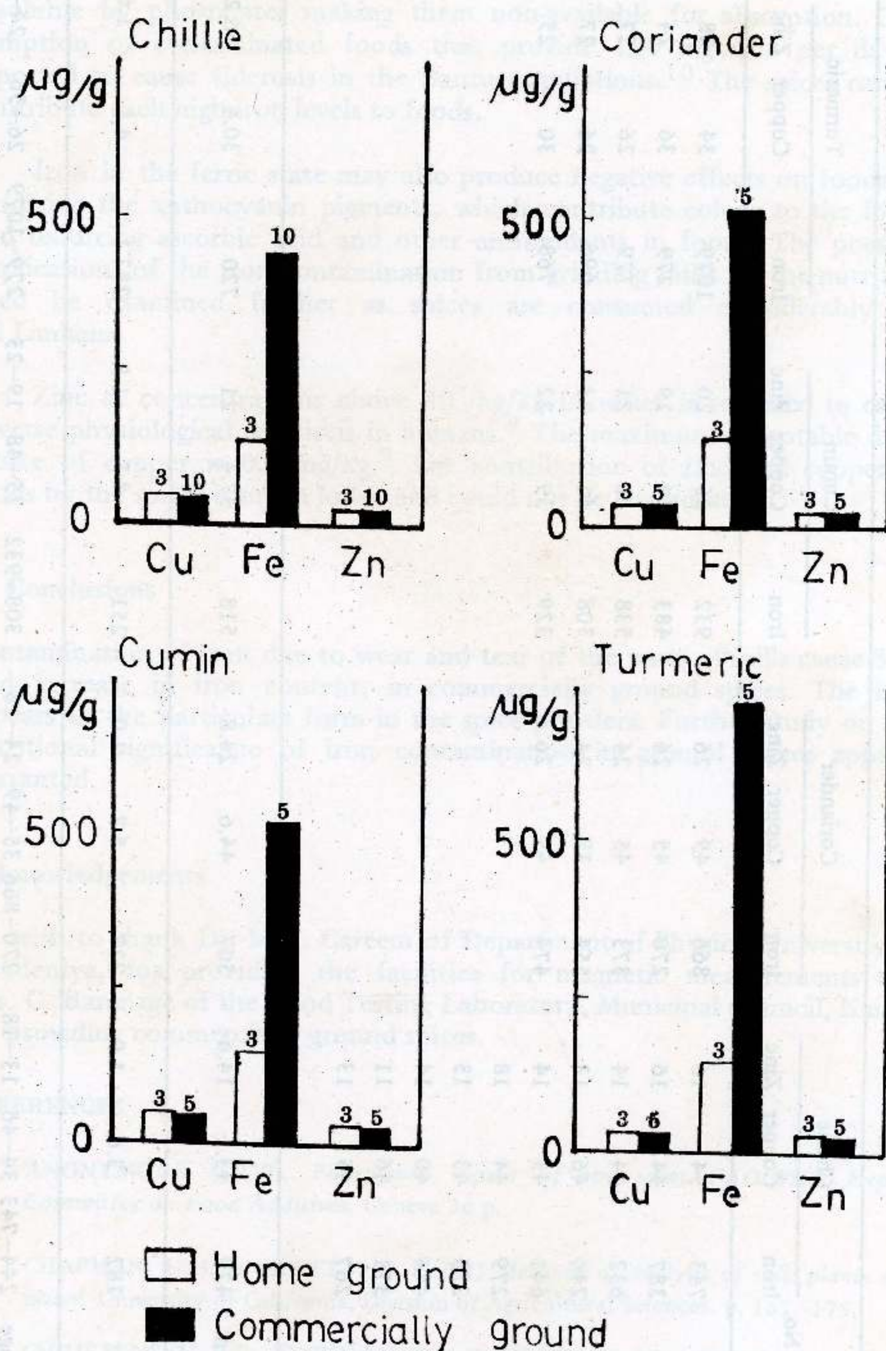

Figure 1. Copper, iron and zinc concentrations (µg/g) in spices ground at home and commercial mills from the same samples of whole spices. Figures on bars refer to the number of samples assayed.

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Table 4. Iron, Copper and Zinc concentrations ($\mu g/g$) in the same chillie, coriander, cumin and turmeric samples ground in different commercial grinders. They are replicates of samples ground by home methods (Table 2).

Mill No.	Chillie			Coriander			Cumin			Turmeric		
	Iron	Copper	Zinc	Iron	Copper	Zinc	Iron	Copper	Zinc	Iron	Copper	Zinc
1	743	34	16	866	49	26	932	25	20	1079	34	28
2	387	44	16	270	45	24	483	34	19	279	36	24
3	632	44	14	373	45	24	538	48	21	779	26	27
4	244	46	13	554	35	24	308	41	23	696	26	27
5	641	45	14	474	49	26	329	39	22	768	30	25
6	279	44	18									
7	275	43	13									
8	444	40	14									
9	371	46	11									
10	267	45	13									
Mean	429	43.1	14.6	507	44.6	24.8	518	37.4	21	720	30.4	26.2
S.D.	182	3.6	1.9	227	5.7	1.0	251	8.5	1.5	287.1	4.5	1.6
Range	244–743	34–46	13–18	270–866	35–49	24–26	308–932	25–48	19–23	279–1079	26–36	24–28

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other hand the iron may be complexed by phytates or oxalates or made insoluble by phosphates making them non-available for absorption. Consumption of contaminated foods that provide 100 mg iron per day is reported to cause siderosis in the Bantu populations.[10] The spices cannot contribute such high iron levels to foods.

Iron in the ferric state may also produce negative effects on foods by oxidizing the anthocyanin pigments, which contribute colour to the foods and oxidizing ascorbic acid and other antioxidants in foods. The possible implications of the iron contamination from grinding mills on the nutrition need be examined further as spices are consumed considerably by Sri Lankans.

Zinc at concentrations above 40 mg/kg in water is reported to cause adverse physiological reactions in humans.[9] The maximum acceptable daily intake of copper is 0.5 mg/kg.[9] The contribution of zinc and copper to foods by the spices is much lower and could not be hazardous.

4. Conclusions

Contamination of iron due to wear and tear of the parts of mills cause 3–5 fold increase in iron content, in commercially ground spices. The iron appears in the particulate form in the spice powders. Further study on the nutritional significance of iron contamination in ground spices appears warranted.

Acknowledgements

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EVAPOTRANSPIRATION REQUIREMENT OF RICE AT MAPALANA IN THE WET ZONE OF SOUTHERN SRI LANKA

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Abstract : A lysimeter experiment was conducted at the Mapalana Research Farm in order to estimate the evapotranspiration (ET) demand of rice in the southern wet zone. The measured values of ET were compared with pan evaporation (EP). The impact of meteorological factors on ET and EP of rice fields were assessed by the multiple regression method. The ET rate of rice varied in the range of 2–15 mm/day. The ET/EP ratio was almost one at transplanting reaching 1.9 at heading stage. The average ET/EP ratio for the entire growth period was 1.39. It was revealed that wind is the most decisive factor governing ET and EP from rice fields.

1. Introduction

Water requirement of a vegetation is generally expressed as a function of the climate, soil and the plant. Hence, the amount of water needed by the crop varies between species, in different locations and seasons.

Rice needs more water to sustain life than most other crops owing to its semi-aquatic nature. Its evapotranspiration rate is about 3 – 4 mm/day during the initial vegetative stages and 5 – 7 mm/day during reproductive to the medium dough stages.[2,5,6] The average evapotranspiration rate in rice growing areas of Asia is about 4 – 9 mm/day.[8]

There are many reports indicating that the highest evapotranspiration rate of rice is at maximum tillering stage or at heading stage.[5,6] It is evident in many locations of South East Asia that the transpiration rate of rice increases consistantly up to the heading stage and then declines.[6] Nevertheless there are deviations from this general trend. For instance, some workers have observed the maximum rates at the tillering stage followed by an almost constant but lower rate in later stages.[4]

Research on water balance studies of rice in Sri Lanka is mostly restricted to the dry zone of the country.[1] Therefore the objective of the present research was to find the average and peak period consumption of water by rice in the Southern wet zone of the Matara District. The influence of climatological factors on evaporation and evapotranspiration from the rice field is also discussed.

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2. Materials and Methods

The experiment was conducted at the University Research Farm, Mapalana, Matara District, during the Yala season (31st May–15th October) 1987.

Mapalana is located in the Agro-ecological region WL2 (Low country wet zone); the rainfall distribution of the area is typically biomodal with an annual precipitation of about 2385 mm. 864 mm of rain is received during April – August and 911 mm in October – January. This constitutes 36.2% and 38.2% of the annual rainfall respectively. The mean air temperature is fairly uniform at around 28°C throughout the year. The relative humidity is usually higher in March – July and lower in January and February. The average relative humidity of the location is about 71.6%.

The predominant soils of the area are Red yellow podzolic soils with strongly mottled subsoils and low humic gley soils.

Plastic containers with closed bottoms (42 cm in height; 40 and 34 cm in diameter at the top and bottom ends) were used in the field lysimeter experiment. The containers were filled with 30 kg of soil taken from a paddy field and buried in the same field.

The soil taken for the experiment is sandy clay loam (clay 24%; silt 16%; and sand 60%), slightly acidic (pH 5.6) with 2.5% of organic matter.

The set up of the lysimeter tanks was a complete randomized block layout with 3 replications. Water losses from the lysimeters were studied under 3 levels of submergence (2.5, 5.0, 10.0 cm.).

Evapotranspiration and evaporation losses from the containers cropped with rice (variety BG 379/2) and bare soil were measured daily using a hook gauge. Three separate tanks were used to measure the evaporation from free water surface. Water level was readjusted to the required depth by adding water daily. Soil temperature of the containers were recorded daily in the morning and afternoon. Phenological observations were conducted weekly to record the growth differences of the plants.

All containers were kept weeded to avoid excess evapotranspiration losses. Basic fertilizer mixture (N:P:K: –5:15:15) was added at the rate of 250 kg/ha. A top dressing of urea was added to the containers at a rate of 23 kg/ha at tillering stage. Second top dressing was given (N:P:K –25:0:17) at the rate of 95 kg/ha at the flowering stage.

Measured evaporation and evapotranspiration data were correlated with the observed meteorological data viz. relative humidity, wind speed, air

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temperature and dew point temperature. The combined effect of meteorological factors on evaporation and evapotranspiration was assessed by the multiple regression method.

3. Results and Discussion

The evapotranspiration demand of transplanted rice varied from 2–15 mm/day (Figure 1). The total evapotranspiration demand for the season was 515, 513, 549 mm. for 2.5, 5.0 and 10.0 cm. submergence levels respectively. This consumption was for the entire growth period excluding the first two weeks in the nursery and the last two weeks prior to harvest. Evapotranspiration rate increased with the increasing depth of submergence. However, the evapotranspiration rates at different levels of submergence appear to be not significant.

In the first, 20 days of growth, evapotranspiration rate was slightly higher compared to the evaporation from bare soil or free water surface (Figure 1). The evapotranspiration gradually increased after 20 days from transplanting, which is probably associated with the growth and development of the assimilatory surface.

Evapotranspiration had a highly significant relationship ($r = 0.93$) with evaporation during the initial stages (up to 30 days from transplanting) and also in the later stages, ($r = 0.89$) (ie. after 80–105 days from transplanting) However, in the middle of the growing season (30–80 days from transplanting) the correlation between evaporation and evapotranspiration was low ($r = 0.35$). This may be due to the high transpiration rate coinciding with larger leaf area.

The ET/EP ratio was almost 01 at transplanting, reaching 1.9 between 40 – 50 days from transplanting (Figure 2). The average ET/EP ratio for the entire period of growth was 1.39.

Rice had two maxima of evapotranspiration in the tillering and heading stages (Figure 1). According to Kung et al. and Grist,[3,4] the rice crop is in great need of water during the maximum tiller number stage and in the heading stage. Nevertheless in most of the experiments the rate of evapotranspiration observed at heading was much greater than the rate at maximum tillering stage.[6] As reported in Thailand[4] rice may have higher peak rate at tillering stage.

The differences of peak rates at tillering and heading phases may be associated with the morphological characteristics of the variety, such as medium to high tillering capacity accompanied with thick erect leaves and lodging resistance. The peak demand of water on the 6th and 7th weeks may

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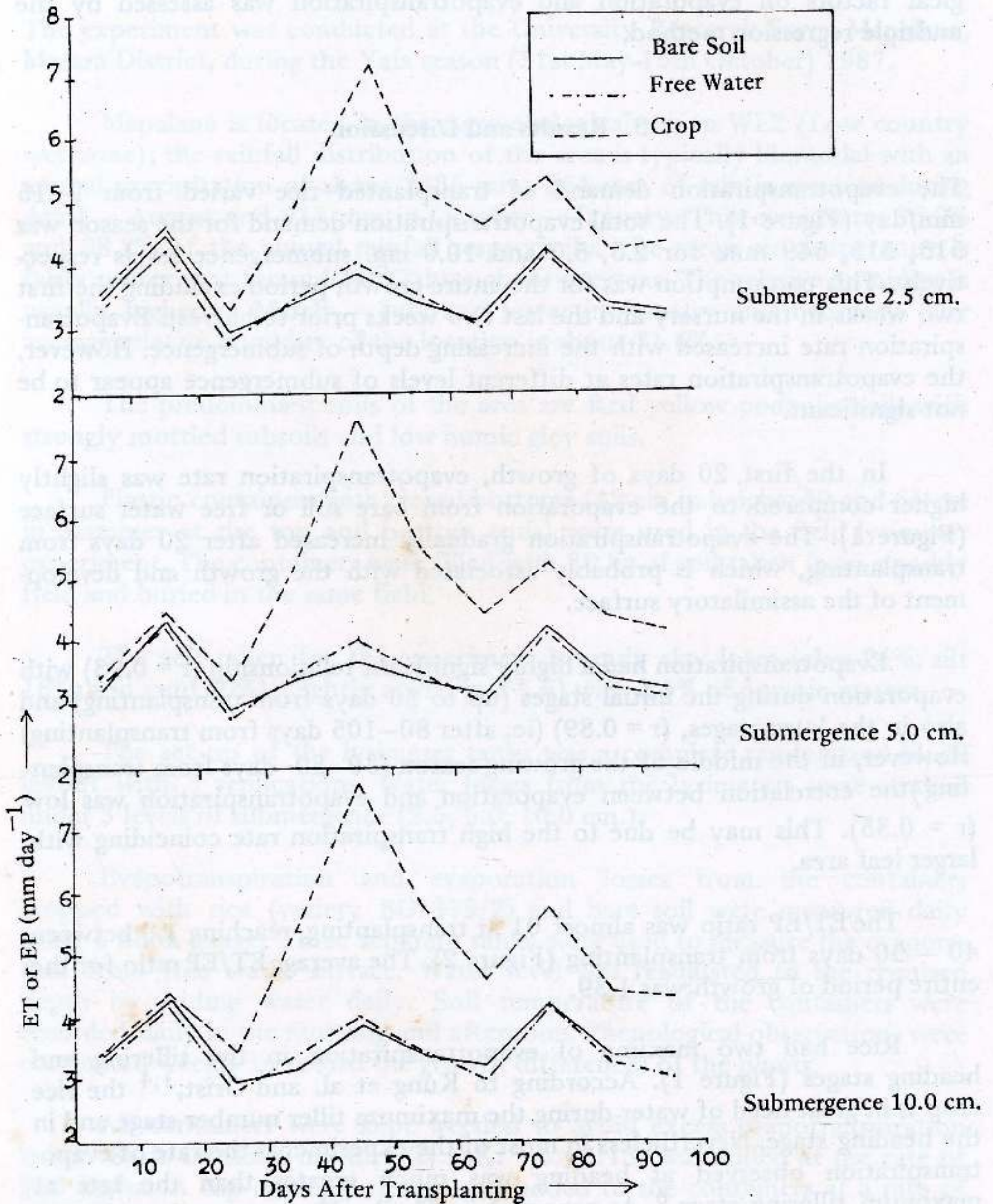

Figure 1. Water losses from the free water surface, bare soil and the cropped soil at various levels of submergence.

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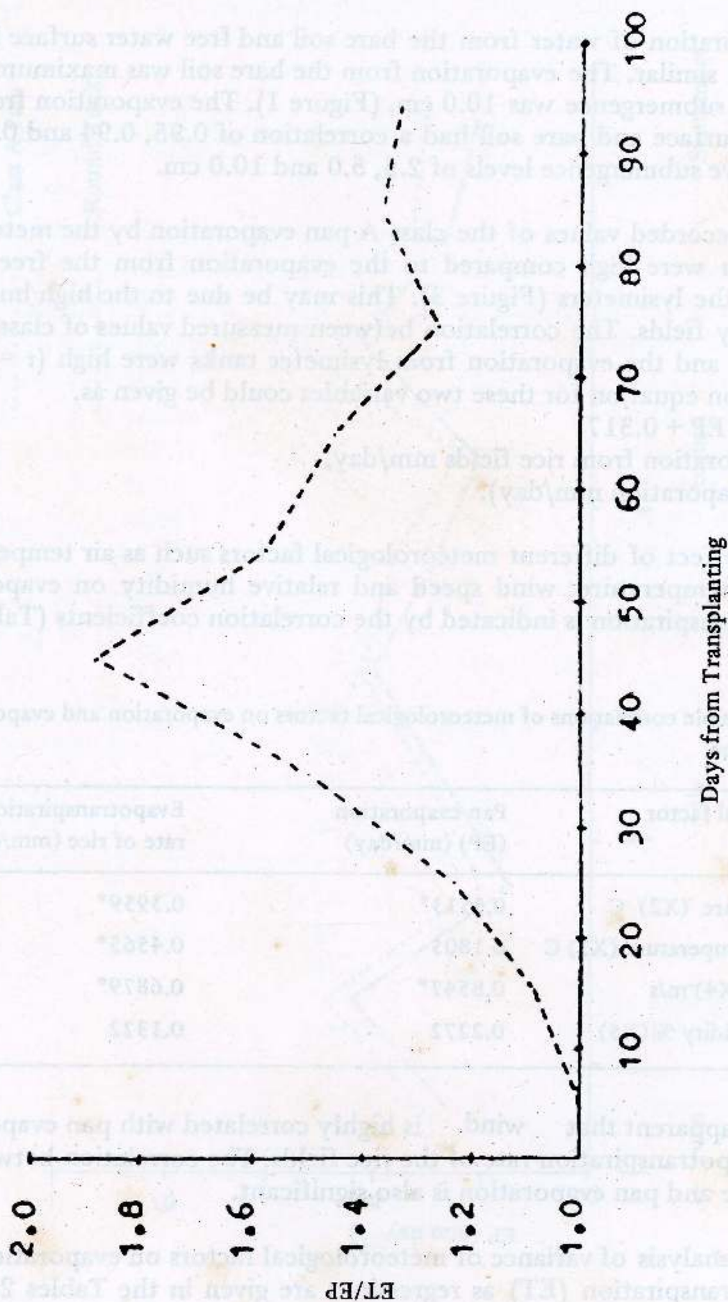

Figure 2. Evapotranspiration to Evaporation Ratios of Paddy

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be associated with the existing meteorological conditions (high wind speed and temperature) and development of tillers and rapid increase in leaf area after the 2nd application of fertilizer late in the 4th week.

Evaporation of water from the bare soil and free water surface in lysimeters were similar. The evaporation from the bare soil was maximum when the level of submergence was 10.0 cm. (Figure 1). The evaporation from the free water surface and bare soil had a correlation of 0.95, 0.94 and 0.91 for the respective submergence levels of 2.5, 5.0 and 10.0 cm.

The recorded values of the class A pan evaporation by the meteorological station were high compared to the evaporation from the free water surface, of the lysimeters (Figure 3). This may be due to the high humidity in the paddy fields. The correlation between measured values of class A pan evaporation and the evaporation from lysimeter tanks were high ($r = 0.92$), the regression equation for these two variables could be given as,

$EW = 0.808\ EP + 0.317$

(EW = Evaporation from rice fields mm/day,
EP = Pan evaporation mm/day).

The effect of different meteorological factors such as air temperature; dew point temperature; wind speed and relative humidity on evaporation and evapotranspiration is indicated by the correlation coefficients (Table 1).

Table 1. Possible correlations of meteorological factors on evaporation and evapotranspiration.

Meteorological factor	Pan evaporation (EP) (mm/day)	Evapotranspiration (ET) rate of rice (mm/day)
Air temperature (X2) C	0.5513*	0.3959*
Dew point temperature (X3) C	0.1805	0.4565*
Wind speed (X4) m/s	0.8547*	0.6879*
Relative humidity % (X5)	0.2272	0.1322

It is apparent that wind is highly correlated with pan evaporation and the evapotranspiration rate of the rice fields. The correlation between air temperature and pan evaporation is also significant.

The analysis of variance of meteorological factors on evaporation (EP) and evapotranspiration (ET) as regressions are given in the Tables 2(a) and

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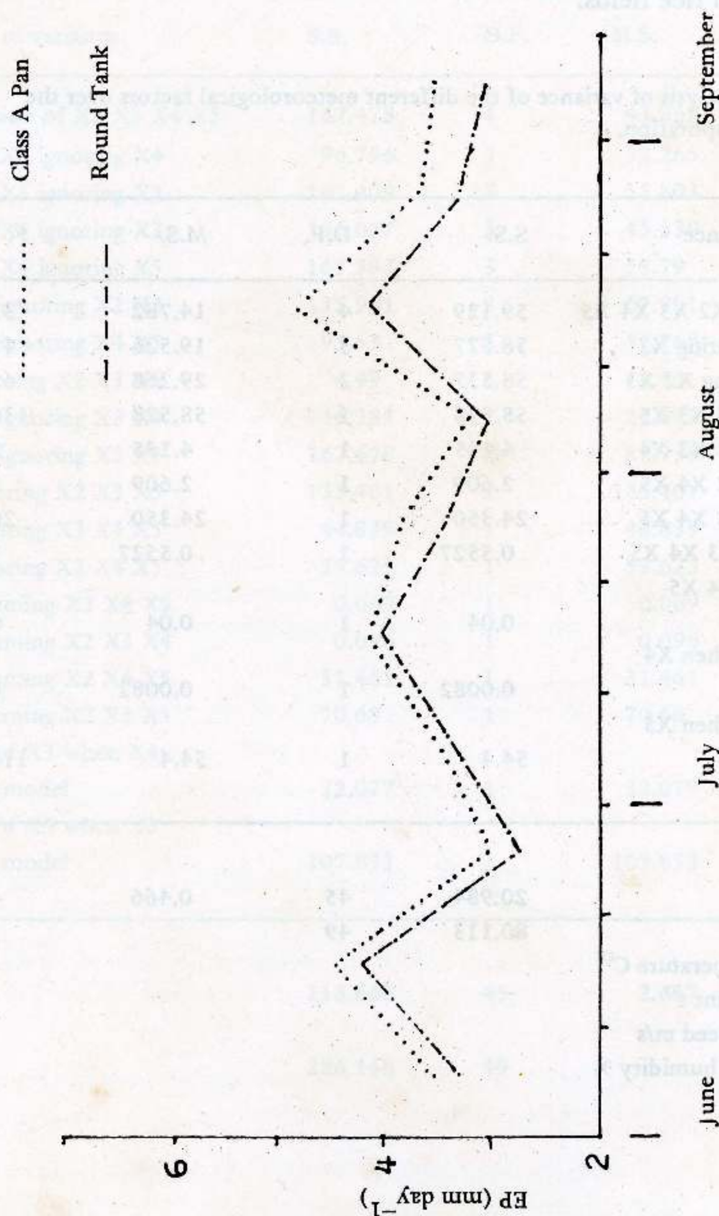

Figure 3. 10 Day Averages of Evaporation from the Class A Pan and the Lysimeters

2 (b). It is clear that the impact of wind wind on evaporation or evapotranspiration is highly significant whether or not air temperature, relative humidity or dew point temperature have an effect on it. Thus it was revealed that wind is the most decisive factor governing pan evaporation and evapotranspiration from rice fields.

Table 2 (a). Analysis of variance of the different meteorological factors over the evaporation.

Source of variance	S.S.	D.F.	M.S.	F. Value
Regression of X2 X3 X4 X5	59.129	4	14.782	31.7*
X3 X4 X5 ignoring X2	58.577	3	19.526	41.7*
X4 X5 ignoring X2 X3	58.537	2	29.268	63.75*
X4 ignoring X2 X3 X5	58.528	1	58.528	130.15*
X5 ignoring X2 X3 X4	4.135	1	4.135	2.012
X3 ignoring X2 X4 X5	2.609	1	2.609	1.016
X2 ignoring X3 X4 X5	24.350	1	24.350	20.96*
X2 assuming X3 X4 X5	0.5527	1	0.5527	1.185
X3 assuming X4 X5 ignoring X2	0.04	1	0.04	0.08
Effect of X5 when X4	0.0082	1	0.0082	0.017
Effect of X4 when X5 in the model	54.4	1	54.4	116.6*
Error	20.984	45	0.466	–
Total	80.113	49		

X2 – air temperature C^{o}

X3 – dew point t^{o}

X4 – wind speed m/s

X5 – relative humidity %

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Table 2 (b). Analysis of variance of the different meteorological factors over the evapotranspiration.

Source of variance	S.S.	D.F.	M.S.	F. value
Regression of X2 X3 X4 X5	167.478	4	41.869	15.877*
X2 X3 X5 ignoring X4	96.796	3	32.265	7.838*
X3 X4 X5 ignoring X2	167.409	3	55.803	21.619*
X2 X4 X5 ignoring X3	136.017	3	45.339	13.892*
X2 X3 X4 ignoring X5	167.382	3	55.79	21.61*
X4 X5 ignoring X2 X3	135.961	2	67.981	21.274*
X2 X3 ignoring X4 X5	95.53	2	47.768	11.778*
X5 ignoring X2 X3 X4	4.99	1	4.99	0.854*
X2 X5 ignoring X3 X4	45.384	2	22.692	4.43*
X3 X4 ignoring X2 X5	167.478	2	83.739	33.094*
X4 ignoring X2 X3 X5	135.401	1	135.401	43.114*
X2 ignoring X3 X4 X5	44.839	1	44.839	8.919*
X3 ignoring X2 X4 X5	59.625	1	59.625	12.635*
X2 assuming X3 X4 X5	0.069	1	0.069	0.026
X5 assuming X2 X3 X4	0.096	1	0.096	0.048
X3 assuming X2 X4 X5	31.461	1	31.461	11.93*
X4 assuming X2 X3 X5	70.68	1	70.68	26.803*
Effect of X3 when X4 in the model	32.077	1	32.077	2.63
Effect of X4 when X3 in the model	107.853	1	107.853	40.89*
Error	118.668	45	2.637	—
Total	286.146	49		

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The regression lines for evaporation (EP) and evapotranspiration (ET) when wind speed (X4) is included to the regression models would be,

EP = 1.4676 + 0.7734 X4 and
ET = 2.4857 X4 − 2.9853
(EP and ET; (mm/day), X4; (m/s) respectively.

These regression lines would be a useful tool in estimating Evaporation (EP), and Evapotranspiration (ET), from the rice fields at Mapalana.

Acknowledgements

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n – TYPE ELECTRICAL CONDUCTIVITY IN CUPROUS OXIDE THIN FILMS

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Abstract : Electrodeposited cuprous oxide thin films were investigated to determine whether their electrical conductivity is n-type or p-type. The experimental results based on the measurements of thermoelectric e.m.f., sheet resistance, dark and light current – voltage characteristics of Cu_2O/Cu_xS and Cu_2O/CuCNS heterojunctions reveal that the electrodeposited cuprous oxide films produce n-type electrical conductivity. These observations are in very good agreement with the previously reported n-type behaviour of the electro-deposited Cu_2O film electrodes in a photoelectrochemical cell.

1. Introduction

Cuprous oxide (Cu_2O) is an attractive material for photovoltatic energy conversion because it has a band gap of 2.0 eV and it is less expensive compared to many semiconducting materials. Experimental and theoretical studies of Cu_2O solar cells have been reported previously.[1,2,3,4] However, the experimentally obtained efficiencies were much less than the theoretically predicted value. Moreover, all the work reported were based on the p-type semiconducting Cu_2O only. Many authors have claimed that the efficiency of Cu_2O solar cells may be improved significantly if a homojunction could be developed provided that the n-type Cu_2O is possible.[5] Nevertheless, n-type photoconductivity of Cu_2O has been reported previously using the photoelectrochemical methods.[7,8] In this investigation, we have undertaken several experiments, other than photoelectro-chemical, to verify the n-type behaviour of the electrodeposited Cu_2O films. Our experimental investigation reveals that the n-type conductivity is possible in electrodeposited Cu_2O films.

2. Experimental

Cuprous oxide thin films were deposited on various metal substrates (Cu, Ti, Pt) using the previously described electrodeposition technique.[8] The metal plates were used as cathodes in an electrochemical cell containing an aqueous solution of 0.01 M $CuSO_4$ with an added few drops of NaOH, while a carbon rod was used as the anode. The electrodeposition was carried out for about 1 hour under the constant current density of 10 mA/cm^2. However, for Cu

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substrates simple dipping of the samples in the electrolyte for about 8 hours was sufficient to produce samples with considerable thickness of Cu_2O films. Most of the measurements in the investigation were carried out for the samples prepared on Cu substrate using the dipping method. However, we have found that the experimental results were the same for all the samples prepared on different metal substrates, but the magnitudes varied somewhat from sample to sample.

Thermoelectric e.m.f measurements were obtained by keeping the substrate of the samples in thermal contact with an electric heater while the film surfaces were being exposed to air. Sheet resistance measurements were made using the conventional four-probe technique. The probes were made of gold plated copper wires and it was also ensured that the I–V characteristics being linear over the range used in these experiments.

Thin copper sulfide (Cu_xS) coatings were deposited on the Cu_2O films by spraying an ammonium sulfide solution (10% by volume) over the surface of Cu_2O films. However, thin CuCNS films were coated by immersing the Cu_2O samples in an aqueous electrolyte of 3M KCNS containing 5% (by volume) of acetic acid for about 2 seconds.[10] For both types of coatings, however, it was observed that the thicknesses of the coatings were crucial and overexposure would not produce the expected junction behaviour. Dark and light current – voltage measurements of the above samples were taken by making electrical contact to the front surface by using a mechanically pressed gold – plated copper wire. Back contact was made to the metal substrate. The light source was a 150 W tungsten – halogen lamp and the light intensity was measured with an International light IL 700 research radiometer.

3. Results and Discussion

Thermoelectric e.m.f measurements were obtained for both types of samples, namely the electrodeposited Cu_2O films on the various metal substrates and the thermally grown Cu_2O films on Cu substrates. Always the thermoelectric e.m.f values for electro-deposited samples were in opposite sign with the thermally grown samples, however, the magnitudes vary from sample to sample. This simple observation suggests that the majority carriers in the electrodeposited Cu_2O films are electrons, compared with the well established result of p-type carriers in the thermally grown Cu_2O films.

It is known that thermally grown Cu_2O films on Cu substrates produce Cu/Cu_2O Schottky contacts at the substrates. In our investigation we have given special attention to this contact effect, because, if the electrodeposited Cu_2O films also produce this effect then the interpretation

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of the heterojunction using the sheet resistance measurements and the photoeffect is questionable. Therefore, all the electrodeposited Cu_2O samples on various metal substrates were subjected to the sheet resistance measurements, and also to tests for the possible photoeffects. We have not observed any significant sheet resistance value, which suggests the shunting through the metal substrate in the absence of a junction, not any photoeffect. This result rules out any possible junction effects that might interfere with our experimental observations.

Sheet resistance values were obtained for the Cu_xS coated Cu_2O films and for the CuCNS coated Cu_2O films. These values were within the range of 500–1000 $\Omega/\square$ for both types of coating. However, the sheet resistance values of Cu_2O, Cu_xS and CuCNS films alone, prepared on the Cu substrates were negligible as mentioned previously. One possible way to explain the increase in sheet resistance is due to the fact that the shunting resistance of the surface layer is increased by the formation of a heterojunction. In general, this is the case if Cu_2O is n-type, since both Cu_xS and CuCNS are p-type.[7,9] Further evidence to the existence of a heterojunction is shown in Figure 1. The dark and light current – voltage behaviour of Cu_2O/Cu_xS junction clearly demonstrates the blocking nature of the junction, as well as the photoeffect. The decrease in the photosignal in the forward bias direction also clearly indicates the existence of a space charge layer at the junction which tends to vanish at forward condition. Although, Figure 1 shows the results of Cu_2O/Cu_xS junction, similar results were obtained for Cu_2O/CuCNS junction as well.

Thermoelectric e.m.f measurements directly suggest that the electrical conductivity of electrodeposited Cu_2O films is n-type. Observations of the possible existence of the heterojunction effects with Cu_xS and CuCNS p-type semiconductors also favour this suggestion. Furthermore, we have an another evidence for the n-type behaviour of Cu_2O films, namely, the observation of the n-type photoconductivity in electrodeposited Cu_2O films in a photoelectrochemical cell.[8] All these experimental evidences lead to the same conclusion that the electrical conductivity of electrodeposited Cu_2O films is n-type. However, the origin of this n-type behaviour is unknown and the possibility of oxygen vacancies and/or the additional copper atoms at the interstitial positions still to be examined.

4. Conclusion

In conclusion, the experimental evidences that we have presented here support the idea of the existence of n-type electrical conductivity in electrodeposited Cu_2O films. We also believe that the heterojunctions Cu_2O/Cu_xS and Cu_2O/CuCNS may be useful in developing inexpensive thin film solar cells. This will be the subject for our future investigations.

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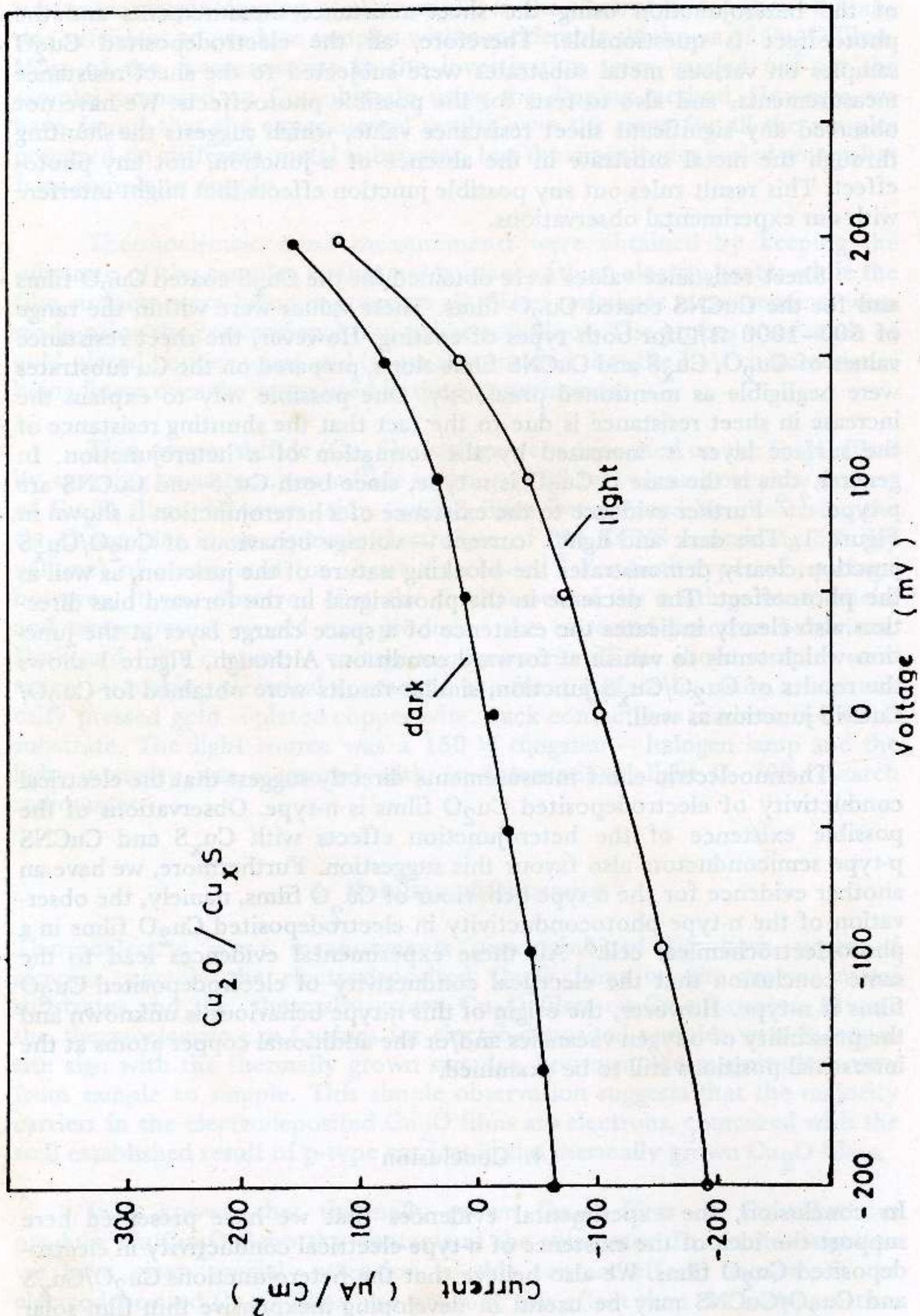

Figure 1. Light and dark current-voltage characteristics of Cu_2O/Cu_xS junction. Light intensity = 50 mW/cm^2

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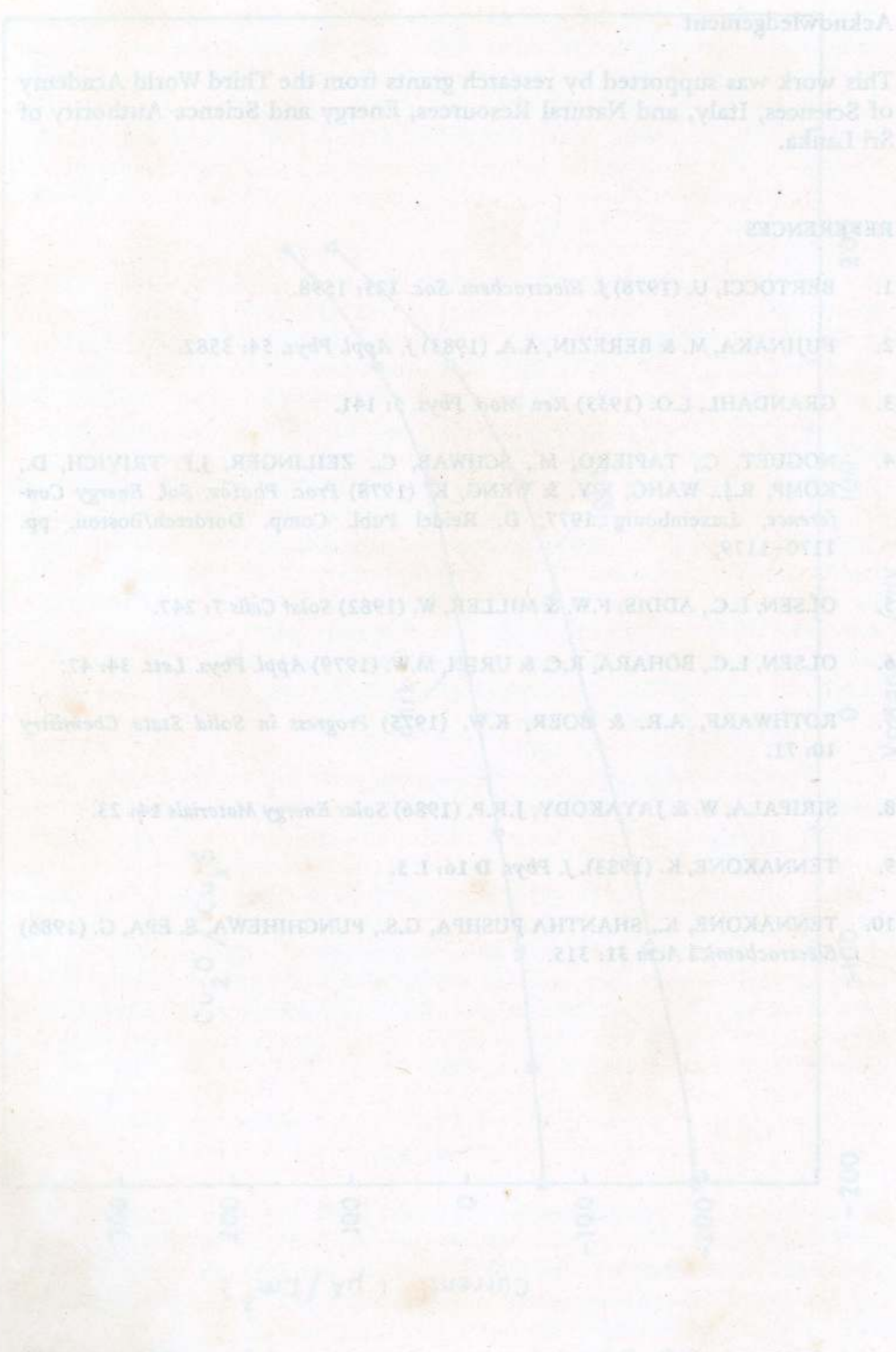

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PALMYRAH PALM WINE 1: MICROBIAL AND BIOCHEMICAL CHANGES

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(Date of receipt : 12 October 1986)
(Date of acceptance : 27 January 1988)

Abstract : The palmyrah palm wine, or 'toddy', is the spontaneously fermented sap of the young and mature inflorescences of both male and female palmyrah *(Borassus flabellifer)* palms. The alcohol content of naturally fermented palmyrah toddy was usually ranging between 5.2 – 5.6% v/v. However, in spite of the lower sugar content in the sap, the toddy from matured female inflorescences contained higher percentages (an average of 6.5% v/v) of alcohol and this increased efficiency of fermentation may be due to either the presence of fermentable substrates other than sugars or to the presence of yeast strains with superior fermentative ability. Studies reveal that the *Saccharomyces* yeasts PY 1 and PY 10 were the best fermentors among those isolated from palmyrah toddy and the yeast *Schizosaccharomyces pombe* PY 7 produced alcohol to a similar extent, but after longer fermentation. It was also found that the efficiency of sucrose to alcohol conversion can be increased upto about 92% by aseptically inoculating *Saccharomyces* yeasts as a monoculture into unfermented sap.

1. Introduction

The main product of the palmyrah palm *Borassus flabellifer* is the sap extracted from the inflorescences by a process known as 'tapping'. This sap can be consumed either in an unfermented stage (sweet toddy/'neera') or in a fermented stage (wine/toddy) or it may be consumed in some processed form such as 'jaggery' or sugar candy.

The term 'fermentation' can be defined in general terms as an oxido-reduction process, where the terminal electron acceptors are organic compounds. Normally, sugars, in an intermediate oxidation state, fulfil this requirement.

The current method of fermentation of palmyrah sap is an uncontrolled spontaneous process, taking place in the collecting pot due to the accumulation and growth of wild yeasts from the air. As the same earthenware pot is used for several successive days for toddy or wine collection, its inner surface gets coated with a layer of yeasts and bacteria.

The fresh, unfermented sap obtained from the inflorescences of palmyrah palm contains 10 – 16.5% w/v sugar, mainly in the form of sucrose.[9] This sucrose initially undergoes hydrolysis to form glucose and

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fructose, due to the action of the enzyme 'invertase' located in the yeast cell wall close to the external surface.[7] Glucose and fructose, transported into the yeast cells, undergo glycolysis via the Embden–Meyerhof–Parnas pathway[2] resulting in the production of ethanol, carbon dioxide and energy. This fermented product is popularly known as 'toddy' or 'palm wine' and the component of interest in this product is ethanol.

The inorganic constituents of toddy samples have received less attention than have the organic components. Nevertheless, the minerals are of considerable significance in fermentation processes and many of them are of significance in human nutrition.[1]

'Toddy' contains a large quantity of wild yeasts and bacteria. The bulked toddy contains about $10^7 - 10^8$ cells of yeasts per ml of the sample. Yeasts belonging to the genus *Saccharomyces* (PY 1 and PY 10), *Schizosaccharomyces* (PY 7) and *Kloeckera* (PY 2) were isolated from the naturally fermented palmyrah palm wine.[11] Most of the bacteria in the palmyrah toddy samples were identified as belonging to the genus *Bacillus.*[10]

As reported by Thirukanesan and Theivendirarajah[12] the method of tapping a palmyrah palm varies with the sex of the palm and age of the inflorescence. In the present study, the average alcohol content in toddy samples obtained by different methods of tapping, viz., 'aripanai', 'vallupanai', 'thattupanai' and 'kaivetty' was determined. The fermenting abilities of the different yeasts (those previously isolated from naturally fermented palmyrah wine) and the changes in sugar and alcohol contents during the fermentation process were also studied.

2. Materials and Methods

2.1 Collection of Materials and Preparation of Experimental Media

2.1.1 Fresh unfermented sap

This was collected in sterile MacCartney bottles by holding the bottle at the tip of the inflorescence for about 3 minutes. The sample was immediately stored at 5°C or was heated in a boiling water bath for 15 minutes to inactivate the microorganisms which may have contaminated the sap. This sample was mainly used to determine the sugar content of unfermented sap.

2.1.2 Sweet toddy

Palmyrah sweet toddy was collected for 14 hours in earthenware pots; the inner surface of these pots were coated with slaked lime.

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The lime used in the collection of sweet toddy was removed initially by sedimentation and later by precipitation as calcium phosphate by adding superphosphate. Precipitation was enhanced by heating to about 40 – 50°C and by centrifugation. This centrifuged, decalcified sweet toddy was a clear, colourless liquid with a pH around 6.5–7. This was sterilized by autoclaving at 15 lb/in ^{2} pressure (121°C) for 15 minutes.

2.1.3 Fermented toddy

This was obtained by collecting the palmyrah sap in earthenware pots by adopting the traditional process of toddy collection. Usually the samples were obtained in mornings after a collection period of about 14 hours.

2.1.4 Peptone-yeast extract-sugar medium

Peptone	3.0 g/l
Yeast extract	5.0 g/l
Glucose &/or sucrose	defined amount
Agar (if necessary)	25.0 g/l

This medium was sterilized by autoclaving at 15 lb/in^2 pressure for 15 minutes.

2.2 Collection and Preparation of Yeast Cultures

Four different yeasts, viz., *Saccharomyces cerevisiae* PY 1, *Kloeckera apiculata* PY 2, *Schizosaccharomyces pombe* PY 7 and *Saccharomyces chevalieri* PY 10 were isolated from the naturally fermented palmyrah toddy.[11] These isolates were transferred into peptone-yeast extract-glucose (4% w/v)–agar slopes and stored at 4°C.

A loopful of the culture in need was transferred into 100 ml portion(s) of sterile peptone-yeast extract-glucose (2% w/v) broth and was allowed to grow for 12 hours; then the sedimented yeast mass was separated by centrifugation, washed well with sterile water and appropriately diluted with sterile 0.85% (w/v) saline. This was used as the starter culture for the experiments.

2.3 Methods

2.3.1 Routine analytical methods

Amount of sugar in a sample was estimated according to the Somogyi's semimicro method.[3] Alcohol content was determined using an ebulliometer or by the specific gravity method.[6]

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Yeast cell count was estimated using a haemocytometer and the pH of the medium was determined using a Phillips PW 9418 pH meter.

2.3.2 Experimental procedures

(a) Alcohol content of naturally fermented palmyrah toddy and the efficiency of the natural fermentation process.

The alcohol content of representative samples of palmyrah toddy collected from different parts of the Jaffna-peninsula was determined after 48 hours of fermentation. Similarly, the sugar content of fresh, unfermented sap samples obtained from the male and female inflorescences of the palmyrah palm was determined by the routine method. The efficiency of the natural fermentation process was calculated on the basis of sucrose to alcohol conversion. About 15 samples of toddy were analysed under each of the different methods of tapping and the results were statistically analysed according to Bailey[5] and are presented in Table 1.

Table 1. Alcohol content of naturally fermented palmyrah toddy and the efficiency of fermentation.

Method of tapping & inflorescence	Mean sugar content of sap % w/v	Expected alcohol content % v/v	Observed alcohol content % v/v	Efficiency of fermentation
'Aripanai' – young male	12.94 p	8.9	5.28 a	59%
'Vallupanai' – matured male	13.33 p	9.19	5.53 a	60%
'Thattupanai' – young female	15.15 q	10.45	5.67 a	54%
'Kaivetty' – matured female	10.89 r	7.52	6.54 b	87%

The values denoted by the different letters p, q & r and a & b are statistically different at 5% level ($p < 0.05$).

Number of experiments: 15 for each method of tapping.

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(b) The fermenting abilities of the isolated yeast and bacterial strains:

The alcohol production by the yeasts mentioned in 2.2 was estimated by separately introducing an innoculum of 10^5 cells/ml of each yeast isolate into

(i) 100 ml portions of palmyrah sweet toddy medium (denoted as PST)
(ii) 100 ml portions of peptone-yeast extract broth having 14% w/v sucrose and 1% w/v glucose (denoted as DF).

The alcohol content in each case was determined using the ebulliometer after 24, 48 and 72 hours of fermentation. As the sugar content of the two media employed differ, the efficiencies of alcohol production by these different yeast isolates can be compared. The maximum alcohol produced by these yeast isolates and their fermentation efficiencies are presented in Table 2.

Table 2. Maximum alcohol production and efficiency of fermentation of the different yeast isolates.

Yeast	Maximum alcohol content % v/v		Efficiency of fermentation	
	PST	DF	PST	DF
Saccharomyces cerevisiae PY 1	8.3	8.0	92.29%	77.56%
Kloeckera apiculata PY 2	0.0	0.32	0.0%	3.10%
Schizosaccharomyces pombe PY 7	7.85	8.0	91.07%	77.56%
Saccharomyces chevalieri PY 10	8.0	7.5	92.81%	72.71%

PST = Palmyrah Sweet Toddy medium
DF = Defined medium (peptone-yeast extract-sugar medium)

2.3.3 *Microbial and Biochemical changes during natural fermentation of palmyrah toddy*

A new, clean clay pot was tied to the palmyrah inflorescence in the evening about 5 p.m. and the sap which dripped from the young female inflorescence into the pot was collected at about 6 a.m. on the following morning. Total and reducing sugar contents, alcohol content, yeast cell number and pH were determined periodically and the results are presented in Figure 1.

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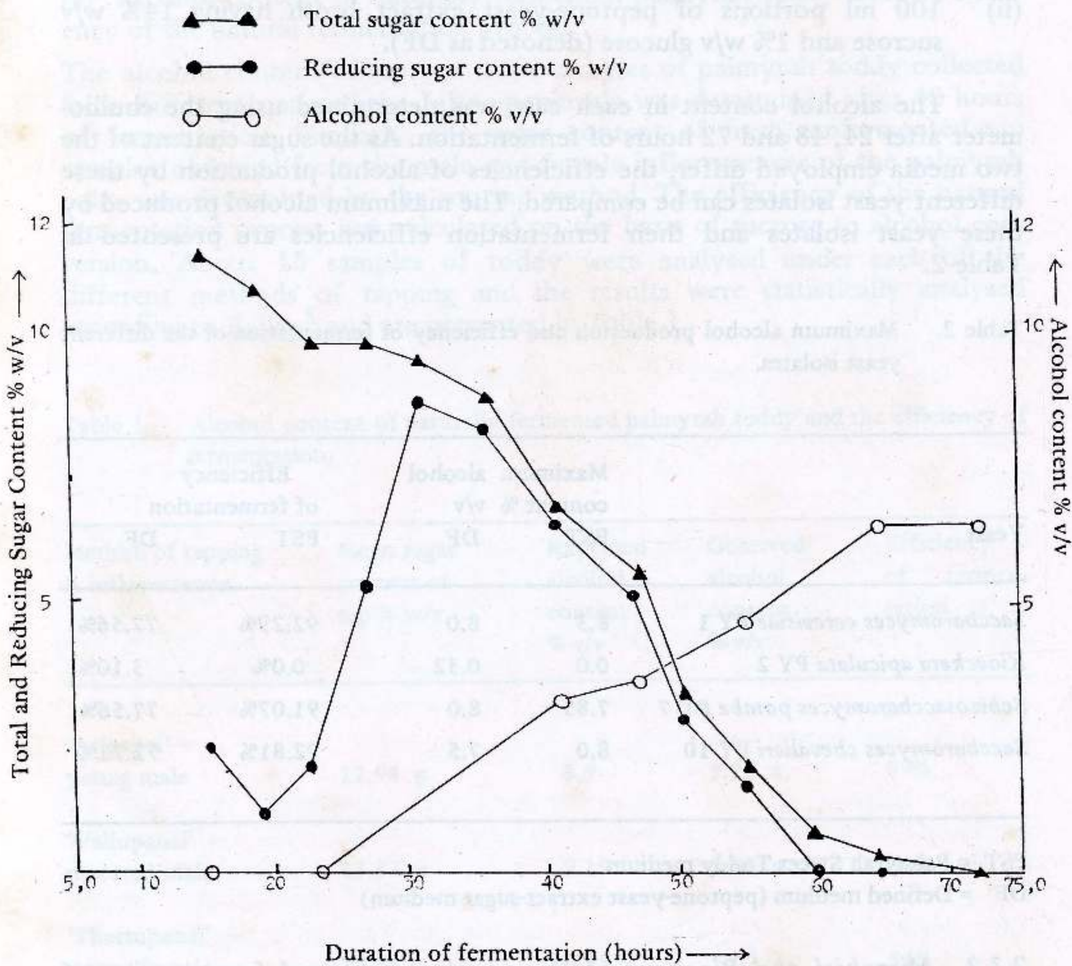

Figure 1. Changes in alcohol content and sugar content during natural fermentation of palmyrah toddy.

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3. Results and Discussion

3.1 Alcohol content of naturally fermented palmyrah toddy and the efficiency of the natural fermentation process

The results (Table 1) showed that the alcohol content of toddy samples collected towards the latter part of the tapping season from matured female inflorescences by the 'kaivetty' method of tapping was significantly more than in the samples obtained by the other three methods of tapping. Contrary to the earlier supposition that the sugar content of sap from matured female inflorescences would be high by comparison, an analysis of total sugar content in unfermented sap obtained by the 'kaivetty' method of tapping indicated that it is significantly lower than the sugar content in saps obtained by the other tapping methods.

As for the conversion of total sugar to alcohol, the toddy obtained from the matured female inflorescences had the highest efficiency of fermentation of about 87%, whereas the efficiency of fermentation in toddy samples obtained by the other three methods of tapping was in the range of 50 – 60%.

This lower efficiency of alcoholic fermentation was anticipated because of the presence of a mixed flora of yeasts and bacteria. The formation of several by-products and the conversion of about 1–2% of the sugars into yeast cell mass also accounts for the low yields of alcohol. In addition, some alcohol may get lost to the environment by entrainment in the carbondioxide, and by evaporation.

In spite of the lower sugar content of the sap, the matured female inflorescences produced toddy samples with higher alcohol contents, clearly indicating that the fermentation process was more efficient in sap derived from matured female inflorescences. This may be due to some difference in the microflora, which, in turn, may be affected by the climate and the composition of the sap. The 'kaivetty' method of tapping is employed at the stage when the female flowers have been fertilized. Consequently the sugars directed to these inflorescences may have been converted into other fermentable substances such as glucosides, amides or aminoacids, for possible use in the processes of fruit maturation and germination. As further development of the fruit is hindered by the tapping process, these substances may yield alcohol by undergoing fermentation. This hypothesis has yet to be supported by further experimental evidence. Also, it may be possible that this variation in sap composition may favour the presence and growth of highly fermentative yeast strains in sap from the matured female inflorescences of the palmyrah palm.

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This fermentation process can be improved under controlled conditions using pure yeast cultures on unfermented sap extracted from the palmyrah inflorescences.

3.2 The fermenting abilities of the isolated yeast and bacterial strains

The results presented in Table 2 indicate that as a general rule, the yeasts isolated from palmyrah toddy ferment and produce alcohol more efficiently in the palmyrah sweet toddy medium than in the defined medium. This may be accounted for by the different compositions of the media; these yeasts, by a process of natural selection over a long period of time, would have adapted to the prevailing environmental and nutritional conditions. The palmyrah palm sap may consist of some additional vitamins and aminoacids which favour the growth of these yeasts and thus enhance alcohol production by them.

The lemon-shaped yeast *Kloeckera apiculata* (PY 2) is incapable of utilizing sucrose either fermentatively or oxidatively. Therefore, the relatively low amounts of reducing sugars in the media (1.4375% w/v and 1% w/v in palmyrah sweet toddy medium and defined medium respectively) would have been used by this particular yeast for the increase in biomass. Hence, there would not be appreciable quantities of reducing sugars left in these media so that they can be fermented by this yeast to produce alcohol.

These studies reveal that the *Saccharomyces* yeasts PY 1 and PY 10 are the best fermentors among those isolated from naturally fermented palmyrah toddy samples. Though the fission yeast *Schizosaccharomyces pombe* PY 7 produced equal quantities of alcohol, the maximum alcohol was obtained after a longer duration of fermentation. This must be attributed to the slower rate of growth of these fission yeasts.

Similar studies were carried out by inoculating palmyrah sweet toddy medium with *Bacillus sphaericus* B 2 , *Bacillus cereus* B 7 and *Bacillus firmus* B 17, all of which were previously isolated from naturally fermented palmyrah toddy. The pH and sugar content of the media were estimated periodically. The results revealed that the presence of bacteria does not significantly affect the pH of the palmyrah toddy but they do utilize a small portion of the reducing sugars present in the medium. It was also found that the growth of these bacteria is somewhat suppressed in media having a pH less than 6.0 at the time of inoculation. The naturally fermented palmyrah wine has a pH in the range of 3.8 – 4.2, which suggests that bacterial contamination and growth take place in the early stages of fermentation. When fresh unfermented palmyrah sap or decalcified, neutral sweet toddy is exposed to the air, the sap or medium becomes turbid and milky in appearance. This is due to the contamination of these media by large

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numbers of bacteria prior to the appearance of yeasts in these samples. The yeasts prefer an acidic medium for growth. The bacteria impart a whitish colour to the palmyrah toddy and may also contribute to its aroma and flavour.

All these bacteria require some aminoacids and vitamins for their growth and may utilize the aminoacids and vitamins found in palmyrah sap thus reducing the nutritive value of toddy formed as a result of the spontaneous fermentation. Also, these bacteria may secrete extracellular substances affecting the growth of yeast and, hence, alcohol production.

3.3 Microbial and Biochemical changes during natural fermentation of palmyrah toddy

The results presented in Figure 1 clearly indicate that the initial step in the alcoholic fermentation process is the hydrolysis of sucrose to reducing sugars. Ethanol production seems to begin only after the formation of appreciable quantities of reducing sugars. The maximum alcohol produced was 6.4% v/v and the pH dropped from 4.4 (after 15 hours of fermentation) to 3.9 after 72 hours of fermentation.

Saccharomyces yeasts were the predominant occupants of natural palmyrah toddy with varying amounts of *Schizosaccharomyces*. Yeasts belonging to the genus *Kloeckera* were observed in the sample at the initial stages of fermentation, but the *Saccharomyces* yeasts overgrew them during the course of fermentation. This observation agrees with those reported by Reed and Peppler[8] and Atputharajah *et al.*[4] This domination of the *Saccharomyces* yeasts may be due to their superior fermentative ability.

It was observed that alcohol production started only after cell density reached its maximum. i.e., the fermentation process followed the growth phase of the yeasts. Therefore, it may be suggested that if a higher density of yeast cells is inoculated, not much sugar will be utilized by the yeasts for their growth and reproduction and most of the sugars in the medium will be converted into alcohol thus increasing the efficiency of fermentation.

These sets of experiments reveal that the efficiency of the fermentation process increases with the use of pure yeast innoculum. However, some other factors, for example, the addition of nutrients, may influence the production of alcohol. Therefore, further experiments are necessary to determine the optimal conditions for maximum production of alcohol from palmyrah sap.

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