

## Seasonal Changes of Wood Formation and Some Characteristics of Heartwood Formation in Teak (*Tectona grandis* L.) Plantation

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### ABSTRACT

Some fundamental investigations of wood and heartwood in plantation grown teak (*Tectona grandis* L.) were carried out. In order to verify the relationship between wood formation and growing conditions, moisture content of soil and the development of leaves were also studied. It was found that moisture content of soil was higher in the rainy season. Since trees started budding in May which was the beginning of the rainy season, those trees grown at the foot of the hill showed earlier development of leaves. As for the time sequence of wood formation using the pinning method, it was revealed that wood formation started after the budding and continued to do so until the end of the rainy season. Parenchyma cells of the outer sapwood had great amount of starch grains as reserve substances and this amount abruptly decreased in the middle and inner part of sapwood. Parallel with the decrease of starch grains, lipid droplets increased. However, in the heartwood, both parenchyma cells and wood fibers contained lipid droplets. Elemental analysis of the black substance in the outer heartwood was tried by SEM-EDXA method and it was revealed that no special element was contributed to the black streak of heartwood.

**Key words :** wood formation, sapwood, heartwood, teak.

### INTRODUCTION

Teak is one of the most important tree species in the world. Although it is the endemic tree species of monsoon area in the southeast Asia, it has been widely planted in the tropical and sub-tropical areas (Donaldson, 1984; Sanwo, 1987; Madoffe and Magheme, 1988).

For the better utilization of teak wood it is necessary to investigate the relationship between wood anatomy and wood qualities including the factors effecting growth conditions. Ferguson (1934) had firstly reported the wood quality. Several

reports have been made on the wood quality of teak (Burmester and Wille, 1975; Bhat *et al.*, 1989). Only few reports, however, were related to the wood formation. Venugopal and Krishnamurthy (1987a, 1987b), for example, studied the seasonal productions of secondary xylem and secondary phloem in twigs. As for the elucidation of xylem formation and heartwood formation in the trunk of teak, much more fundamental studies are necessary.

In this report, therefore, preliminary investigations on the seasonal features of xylem formation and some characteristics of heartwood formation was carried out to get the fundamental

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information being useful for teak wood utilization.

To verify the relationship between tree growth and wood formation, it was important to investigate the photosynthetic ability of leaves and water condition, the seasonal development of leaves and moisture of soil were also measured.

The topography of plantation site is another important factor. In this experiment, therefore, the trees from the ridge, the middle and the foot of the hill were selected from a plantation in the northern Thailand.

## MATERIALS AND METHODS

### 1. Sample trees and the dates of field experiments

An afforestation site of 22-year old teak trees was selected in northern Thailand (Uttaradit province) which situated in the typical monsoon area. To investigate the relationship between tree growth and topography, two sample trees from sites, on the ridge, the middle and the foot of the hill were used for experiments. The results, however, are shown only the trees grown at the ridge and at the foot. The data of the middle of the hill showed basically the intermediate values between the ridge

and the foot. The height of major trees were between 15 to 20m. and the diameter at breast height ranged between 20 to 25cm.

The field experiments were carried out at different times from the middle of 1990 to the middle of 1991. The experiment dates were as follows; Jul. 18, Sep. 26, Nov. 20, Dec. 19 (1990), Jan 10, Mar. 14, Apr. 2 and May 19 (1991). Trees were felled on July 10 and 11 (1991).

### 2. Methods

#### 2.1 Measurement of soil moisture content

The moisture contents of soil relating to tree growth were investigated seasonally. The results were expressed by percentage of moisture based on the dry weight of soil.

#### 2.2 Measurement of leaf-area

To know the seasonal characteristics of the major photosynthetic organs, the seasonal changes of leaf area were investigated.

To measure the leaf-area the photographs of the crown were taken vertically using a fish-eye lens at the same spot in the forest on dry and wet season. The area was calculated using an image analyzing system (Kanazawa and Ishizuka, 1990). Two examples of the fish-eye images are shown in Fig. 1. Phenological changes of leaves were also



**Figure 1** Photographs of the canopy taken by a fish-eye lens. (a) May (dry season), (b) July (rainy season).

observed at some branches.

### 2.3 Measurement of moisture content of wood

The radial distribution of moisture contents from bark to pith at the time of felling were investigated using the wood blocks taken from the disk at breast height. Fresh wood blocks were first measured. The percentage of moisture content based on dry weight of wood was calculated after 24 hrs. drying in an oven set at 105°C.

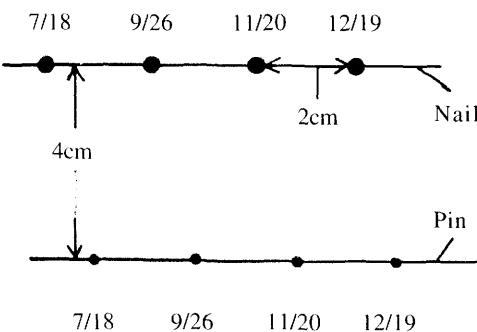
### 2.4 Investigation of seasonal changes of wood formation by pinning method

The pinning method (Shimaji, and Nagatuka., 1971; Shiokura, 1989) was applied to get the seasonal characteristics of wood formation. A large pin (400  $\mu$  in diameter) and a nail (2mm in diameter) were used. The size of the pin is crucial in order to make a successful insertion through the cambial region of the tropical tree which is known to have thick bark and hard wood. However hardness of teak wood is a medium class and the bark is not so thick, it is expected that both the pin and the nail can mark the cambial region accurately.

Schematic represents pinning points is shown in Figure 2.

### 2.5 Microscopy

Fresh wood blocks taken just after felling were fixed with 3% glutaraldehyde. Transverse,



**Figure 2** Schematic representation of the pinning points.

radial and tangential sections of 20  $\mu$  in thickness were cut using sliding microtome. After That staining methods were applied for light microscopy. (I) safranin and light green for general anatomy, (ii) Sudan IV for lipid droplets, and (iii) I<sub>2</sub>KI for starch grains. Unstained sections were also prepared.

### 2.6 Elemental analysis of heartwood substances by an analytical electron microscopy

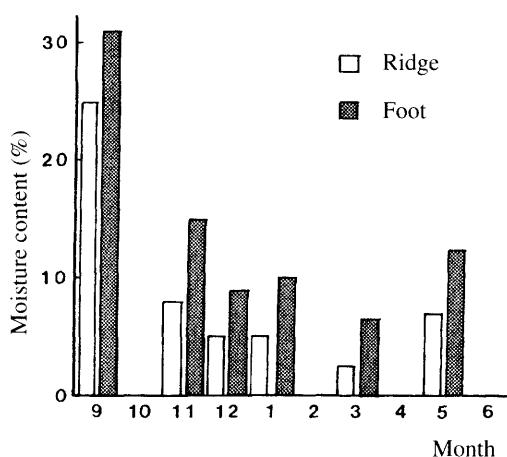
The heartwood color of teak is basically yellowish brown with black streaks. In this report elemental analysis was tried to investigate the chemical nature of the black streak in heartwood region. Radial sections from sapwood and heartwood fixed on stabs were coated with carbon to prevent charging of electrons and investigated by SEM-EDXA technique (Model : JSM-T330A, EDAX 9100/70, 20kV). The illuminating current was set at  $0.5 \times 10^{-9}$  amperes and measuring time was 75 live seconds.

## RESULTS AND DISCUSSION

### 1. The relationship between seasonal changes of soil moisture content and topography

The forest in northern Thailand where experiments were carried out belong to tropical monsoon area. It is, therefore, clearly divided into dry season and rainy season. Rainy season starts from May to June and November is the end of rainy season.

The change of soil moisture content is shown in Figure 3. The moisture content of rainy season, especially at the end of the season, shows high percentage. When comparing the ridge and the foot, the moisture contents of the foot show higher values than those of the ridge without exception. This tendency is quite reasonable result. The characteristics of soil moisture content are considered to have close relationship with tree growth.



**Figure 3** Seasonal changes of the moisture content of soil.

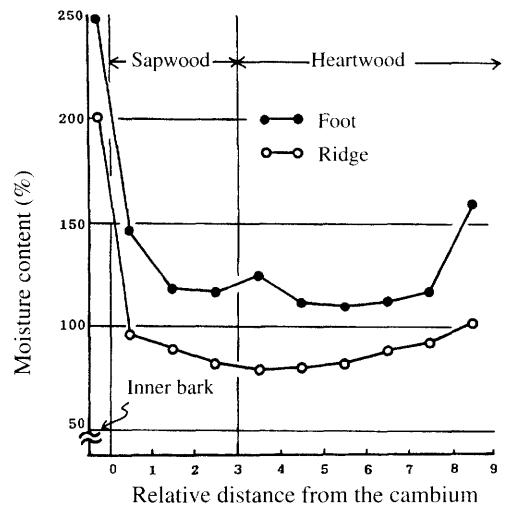
## 2. Moisture content of wood

To consider the effect of soil moisture to tree growth the measurement of moisture content of wood was tried at the time of felling. Seasonal fluctuations of wood moisture content will be investigated in the future.

Figure 4 shows radial changes in moisture content at the breast height of trees from the ridge and the foot. Each value is the average of two sample trees. The abscissa of the figure, moreover, is shown by relative distance based on the ratio of sapwood and heartwood widths.

From Figure 4 the values of the foot are constantly higher than those of the ridge. This tendency is good coincidence with the data of soil moisture. Tissues in bark and wood reserve much amount of water when the soil has high amount of water.

In radial direction the moisture contents of inner bark and outer sapwood show high values. The moisture contents of another part of sapwood and heartwood show rather constant values with the exception in the inner part of heartwood near pith.



**Figure 4** Radial changes of the moisture content of wood from bark to pith.

In ring-porous broad-leaved trees, only outer part of sapwood has the active water transportation in a tree (Krahmer and Kozlowski, 1979). The physiological role of the water in heartwood is still unknown though Sauter (1966) suggested the role of heartwood water to be the water reservoir when the deficiency of water occurs.

From the view point of wood quality the moisture content of heartwood is considered to relate with heartwood color. In Japanese cedar Yazawa and Fukazawa (1956) reported the relationship between heartwood color and moisture content in which higher moisture related with darker color of heartwood. To aim for better utilization of teak, further investigation of the relationship between moisture content and wood color is of essential.

## 3. Seasonal changes of leaves

Carbohydrates which are necessary for wood formation and heartwood formation are, of course, originated from photosynthesis. Phenological observation of leaves and the measurement of the amount of leaves were carried out.

Table 1 shows the result of seasonal change of leaf-area measurement using fish-eye lens method. Data are expressed as relative opening ratio. That is, if the value is smaller, it means large amount of leaves. Moreover, the data of March, in which trees were bare of leaves was fixed as 1.0.

Table 1 indicates that May is the start of budding, and in July trees almost fully developed leaves. These results were supported by the phenological observation of leaves.

Figure 6 shows a light micrograph of a transverse section around a cambial zone at the time of pinning. As Nobuchi *et al.* (1993) investigated pinning parts minutely in Japanese cedar the position of cambial initials and the differentiating zone at the time of pinning were marked in the tissue. The tissue indicated by arrow heads in Figure 6 shows the estimated cambial initials at the time of pinning and the arrows show the estimated position of the initiation of S1 cell

**Table 1** Seasonal changes of relative opening ratio by fish-eye image method.

Plot	Mar.	May	Jul.	Dec.	Jan.
Ridge	1.0	0.97	0.23	0.53	0.88
Foot	1.0	0.98	0.21	0.41	0.48

When comparing trees of the ridge and the foot, trees in the foot started budding earlier and cast leaves later. This result was in good coincidence with the data of soil moisture content in Fig. 3.

#### 4. Seasonal characteristics of wood formation

The anatomical features of the tissues marked by pin and nail insertions were first compared. In both cases cambial zone at the time of pinning could be marked in the tissue. In the case of pin insertion, however, the tip of a pin occasionally did not enter into the cambial region because of the thick and hard bark. To apply the pinning method to the wide varieties of tropical trees including Dipterocarpaceae, marking of cambial zone by a nail was considered to be better. In this report, therefore, the tissues marked by a nail were used for the investigation.

Figure 5 shows the transverse section of a nail driving part. Dark colored parts including wound tissue are developed on both sides of pinning part.

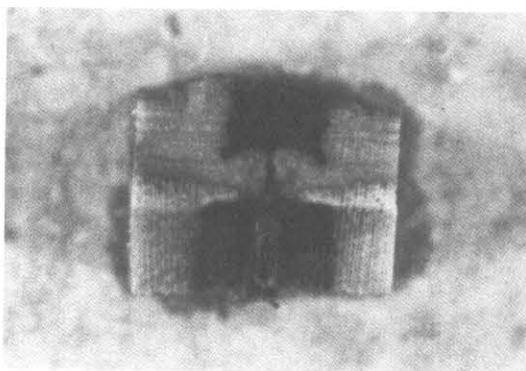
wall layer formation at the time of pinning.

The distance between the estimated cambial initials and the latest annual ring boundary was measured under a light microscope.

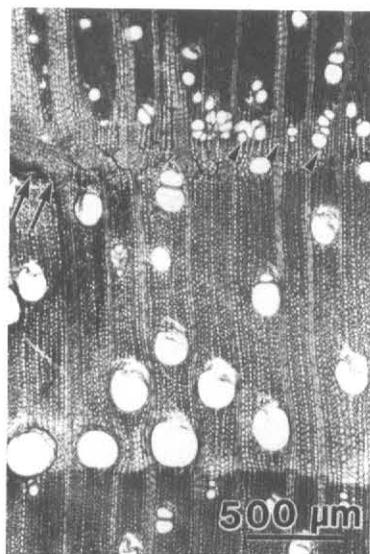
Results were shown as percentages calculated based on the current annual ring width. Figure 7 shows the seasonal feature of the wood formation. Both data from the ridge and the foot are shown in Figure 7. The periods of dry season and rainy season are also indicated in Figure 7.

In Figure 7 only the trace amount of wood formation occurred in May in which trees clearly started budding (Table 1). The development of leaves is considered to occur prior to wood formation.

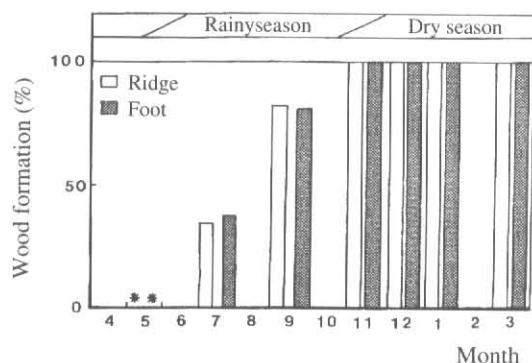
The formation of wood conspicuously progressed in rainy season. Parallel with the start of dry season wood formation stops. This rhythm is the cause of the annual ring formation in teak. It is the future research point to investigate whether teak planted in the region of much amount of precipitation has clear annual ring structure or not.



**Figure 5** A transverse section of a block showing pinning injury and wound tissue.



**Figure 6** A light micrograph of the cross section of wound tissue. Arrow heads show the estimated cambial initials and arrows indicate the initiation of S1 cell wall layer formation at the time of pinning.



**Figure 7** Seasonal changes of wood formation. Asterisks show the trace amount of wood formation.

No difference of wood formation between trees from the foot and the ridge was observed in this experiment. Further minute investigation of wood formation especially in the early stage of wood formation (May, June) and the late stage (October, November) is necessary to clear the differences.

##### 5. Characteristics of heartwood formation

In general the heartwood color of teak is yellowish brown and the boundary between sapwood and heartwood is distinct. It is another characteristic that heartwood has black streaks (Figure 8). In this report cytological observation was mainly carried out.

Figure 9 shows a radial section of outer sapwood stained with I<sub>2</sub>KI. In outer sapwood much amount of starch grains were observed both in ray and axial parenchyma cells.

In middle and inner sapwood the amount of starch grains abruptly decreased. Parallel with the decrease of starch, lipid droplets stained with Sudan IV increased (Figure 10).

In outer heartwood droplets of lipidic nature increased much more than in inner sapwood. These

droplets were observed not only in parenchyma cells but also in wood fibers (Figure 11).

The heartwood of teak, in general, has oily or waxy nature. The droplets stained with Sudan IV in heartwood region are considered to be one of the causes of waxy nature of teak heartwood.

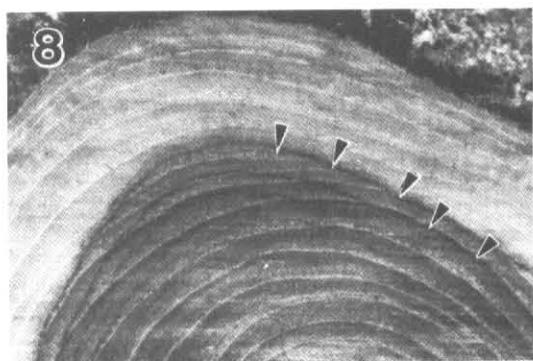
Datta and Kumar (1987) reported the histochemical studies of transition from sapwood to heartwood in teak using branches. In their report the result of starch is the same tendency as the present study but the result of lipids is different from our data and lipid droplets stained with Sudan B were absent in heartwood. One of the reasons of incoincidence is the difference in specimens used, i.e. they used branches. In this experiment all of the sample trees had lipid droplets in heart wood. It is, therefore, believed that teak heartwood has large amount of lipid droplets.

The characteristics of black streaks in heartwood were investigated. Figure 12 shows an unstained section of the outermost heartwood. Black substance existing unhomogeneously in the tissue is considered to be the main cause for the black streaks in heartwood.

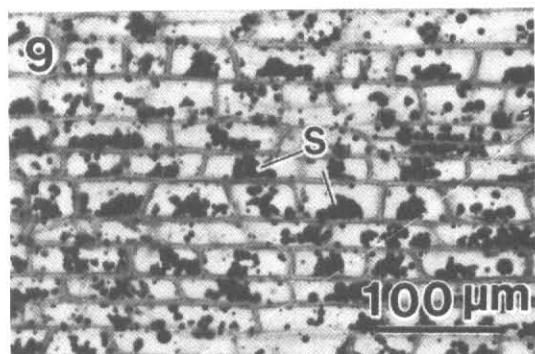
The black substance did not exist in sapwood but only in heartwood. The black material, especially the formation of this material was considered to have close relation with heartwood formation.

In heartwood area this material decreased from outer to inner. One of the possible reasons of this tendency was because the changes in chemical nature from outer to inner heartwood. Another possibility is the migration of the material from parenchyma cells to wood fibers and vessels.

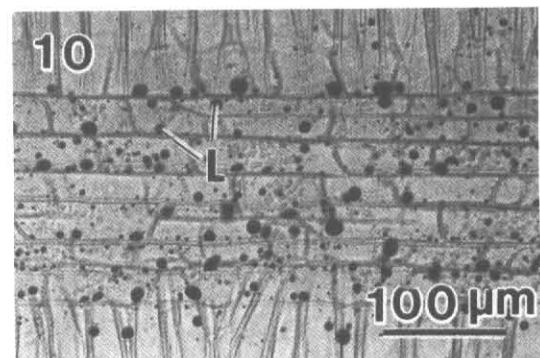
To investigate one of the characteristics of this material EDXA study was carried out. Figure 13 shows the result of elemental analysis of three parts, sapwood, heartwood with black streak and heartwood without black streak (control). Measurement was carried out only on ray parenchyma cells.



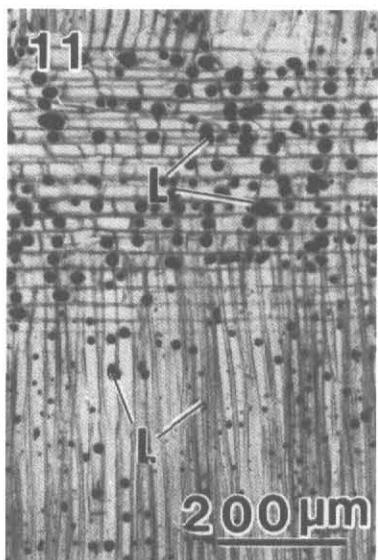
**Figure 8** A transverse section of a trunk indicating sapwood and heartwood. Arrow heads show the black streaks in heartwood area.



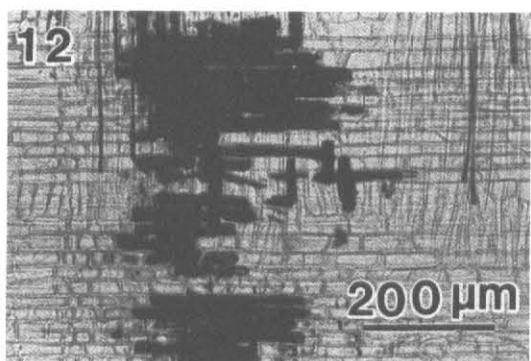
**Figure 9** A light micrograph of a radial section from outer sapwood showing starch grains (S) in ray parenchyma cells.



**Figure 10** A light micrograph of a radial section in inner sapwood showing lipid droplets (L) in ray parenchyma cells.



**Figure 11** A light micrograph of a radial section in outerheartwood showing lipid droplets in ray and axial parenchyma cells and wood fibers.

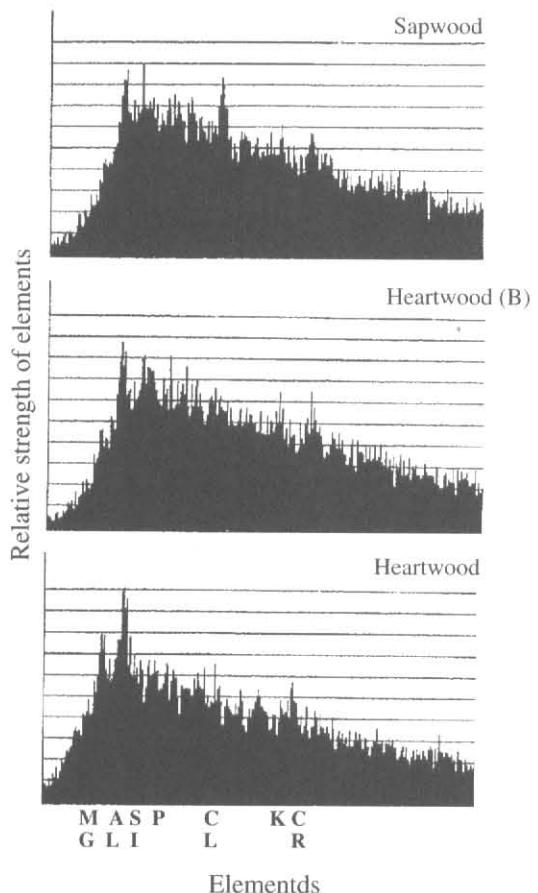


**Figure 12** A light micrograph of a radial section in the outermost heartwood indicating the black substance in ray parenchyma cells.

It was considered that no special element, at least no special heavy metal, concerned with black streak.

Although chemical analysis of this material is necessary, the main component of this material is considered to be organic substances biosynthesized in the transitional zone from sapwood to heartwood.

Based on the preliminary investigation of this report studies of the seasonal characteristics of wood formation and heartwood formation of teak will be continued.



**Figure 13** Three patterns of SEM-EDXA study in sapwood, heartwood with black substance (B) and heartwood without black substance.

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## Bacterial Diversity of Historical Monuments in Thailand and Their Roles on Biodeterioration

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and Chiraporn Aranyanak<sup>2</sup>

### ABSTRACT

Studies on bacterial diversity and distribution on monuments in Thailand were done at three important Historical Parks namely : Sukhothai; Srisutchanalai and Srithep. The representative sites of each Park were Wat Mahathat and Wat Srichum; Wat Changlom and Wat Suwannakeeree and Plang Srithep and Plang Songpeenong, respectively. Total bacterial counts of the two monuments (Sukhothai and Srithep) were found ranging from  $1.79 \times 10^5$  to  $35.58 \times 10^5$  cells/g sample whereas total actinomycete counts were found ranging from  $0.06 \times 10^5$  to  $18.9 \times 10^5$  cells/g sample. Diversity of bacteria and actinomycetes of the three monuments revealed that there were slightly differences in the dominant bacterial and actinomycete genera of each monument. The dominant bacterial genera were *Bacillus*, *Pseudomonas*, *Nitrosomonas*, *Nitrosococcus*, *Nitrobacter* and *Micrococcus*, whereas the dominant actinomycete genera were *Nocardia*, *Micropolyspora*, *Micromonospora*, *Microellobosporium* and *Thermonospora*.

The isolated bacteria and actinomycetes were studied for their roles on the biodeterioration of the monuments. Three important deteriorating factors were revealed : Formation of extracellular water-soluble pigments from certain species of bacteria and actinomycetes with significant amount of dark brown, blue-green, grey, yellow, pink and violet pigments; were formed. Production of extracellular acid, and direct assimilation of building materials by bacteria and actinomycetes. These studies have proved that bacteria and actinomycetes are a couple of the most deleterious biological factors in the deterioration of historical monuments in Thailand.

**Key words** : bacterial diversity, monuments, biodeterioration

### INTRODUCTION

Various historical monuments which are the national precious cultural properties, have been facing the problem of deterioration. In this matter, microbial deterioration is considered as one of the most important direct and indirect factors. Since

there are quite a few scientific researches reported on this subject in Thailand, therefore the objectives of this study are studying and exploration of :

1. Diversity and distribution of bacterial and actinomycete species on the monuments
2. Major roles of the dominant species on biodeterioration of the monuments.

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