EFFECTS OF LOW CONCENTRATIONS OF SO_2 AND NO_X ON THE PHOTOSYNTHETIC CHARACTERISTICS OF *PONGAMIA PINNATA*

E. Philip

Forest Research Institute Malaysia, 52109 Kepong, Kuala Lumpur, Malaysia

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PHILIP, E. 2002. Effects of low concentrations of SO_2 and NO_x on the photosynthetic characteristics of *Pongamia pinnata*. A two-year study was conducted in the urban environment of Shah Alam and Pangkor Laut (both located in Malaysia) to evaluate plant responses to urban air pollution. *Pongamia pinnata* was chosen to address the issue of regular leaf fall patterns in urban areas. Changes in plant performance were considered in relation to the status of ambient air quality in the study areas. Reductions in photosynthetic rates, stomatal conductance, carboxylation efficiency and quantum yield for CO_2 were correlated with air pollution concentrations at these sites. The study showed that the SO_2 and NO_x reduced both stomatal conductance and photosynthetic rates. Changes in stomatal conductance have only a very small quantitative effect on the rate of CO_2 uptake.

Key words: Photosynthesis rates - stomatal conductance - air pollution

PHILIP, E. 2002. Kesan kepekatan rendah SO_2 dan NO_x terhadap ciri fotosintesis *Pongamia pinnata*. Satu kajian telah dijalankan untuk menilai kesan pencemaran udara terhadap pertumbuhan pokok di Shah Alam dan Pangkor Laut (di Malaysia). *Pongamia pinnata* telah dipilih kerana kekerapan spesies ini menggugur daun di kawasan bandar. Perubahan dalam pertumbuhan pokok telah dikaji dengan membandingkan status kualiti udara di kawasan kajian. Pengurangan dalam kadar fotosintesis, konduksi stomata, kecekapan pengkarboksilan dan hasil kuantum untuk CO_2 telah dibandingkan dengan kepekatan pencemaran udara. Kajian menunjukkan bahawa SO_2 dan NO_x menurunkan konduksi stomata dan kadar fotosintesis. Perubahan dalam konduksi stomata mempunyai kesan kuantitatif yang kecil ke atas kadar pengambilan CO_2.

Introduction

In older towns and villages in Malaysia, trees like *Pongamia pinnata*, or mempari as it is locally known, is a popular wayside tree that has been planted for many years. It has a shiny light green crown and a smooth bark that is dull grey to pinkish brown and provides excellent shade in playgrounds and along the roadside (Corner 1986).

Historically, *P. pinnata* has a good track record that credited it as amongst the popular choice when greening programmes were initiated some two decades ago in Malaysia. However, of late, this tree has fallen out of favour as a potential landscaping tree. The reason being it sheds its leaves very often, twice in seven months (Philip & Barakhbah 1994). *Pongamia pinnata* grown in urban area has clear defoliation, with 60–75% of the leaves lost. On the other hand, *P. pinnata* in natural habitat has simultaneous leaf exchange, in which defoliation and flushing occurred together during dry season. The differed leaf fall patterns might be due to air pollution (Philip & Barakhbah 1994).

Reduction in physiological parameters occurs well before visible injuries on leaves are perceived by the naked eye (Keller 1984). Physiological bioindication of air pollutants by *P. pinnata* may, therefore, be of value and have been investigated. It was reported that the tolerance of a species to pollutants is determined either by the stomatal conductance of the leaf or biochemical processes occurring inside the leaf tissues (Mansfield 1973, Thorne & Hanson 1976). Hence, a good understanding of the response of stomata to air pollutants is essential since this is the major pathway of gas flux into leaves (Fujinuma *et al.* 1988).

The effect of SO_2 on stomatal conductance is a paradox. Reports have indicated both an increase as well as a reduction in stomatal conductance when exposed to SO_2 (Majernik & Mansfield 1970, Mansfield & Majernik 1970, Winner & Mooney 1980, Anonymous 1982, Darrall 1989). Attempts have been made to distinguish between stomatal and non-stomatal effects of gaseous pollutants on CO_2 exchange, with more reports suggesting the latter (Furukawa & Totsuka 1979, Furukawa *et al.* 1980, Kumar & Dubey 1998).

On the other hand, reports have indicated that SO_2, acting alone or in combination with NO_x, have caused reductions in the rate of photosynthesis and in the growth of plants in polluted environments (Carlson 1983).

The present study was undertaken with the basic objective of quantifying the changes in the photosynthetic capacity of *P. pinnata* grown in a polluted versus a relatively clean site.

Materials and methods

Study area

The research was conducted at two sites; Shah Alam and Pangkor Laut. Shah Alam is a well-planned satellite town consisting of industrial, administrative, cultural and residential areas. The trees are located in the compound of the Sultan Salahuddin Abdul Aziz Shah Mosque, in the centre of Shah Alam. On the other hand, Pangkor Laut is a private island resort located off the coast of Straits of Malacca. This island is the natural habitat of *P. pinnata.*

Growing conditions at these sites were similar. The mean air temperature, relative humidity and rainfall at both these sites are uniform and not statistically different. Long-term annual average precipitation is 1446.3 and 1800.8 mm for Pangkor Laut and Shah Alam respectively. Average 24-hour mean temperature is 26.7 °C at both sites. Relative humidity is 79.9% at the former and 83.5% at the latter. Except for Mg and Ca, the other chemical properties appear to be similar between the two sites (Table 1). Soil at both places was weathered and heavily compacted. *Pongamia pinnata* can grow on sandy and heavy swelling clay soils (Oyen 1997). The foliar Ca and Mg contents were of no significant different between the two sites (Philip 1998).

Table 1 Soil properties on study sites where *Pongamia pinnata* are located in Shah Alam and Pangkor Laut

Parameter	Control	Polluted
Soil texture	Sandy clay loam	Sandy clay loam
Nitrogen (%)	0.33	0.37
Potassium (meq/100 g)	0.10	0.10
Magnesium (meq/100 g)	0.30	0.10*
Calcium (meq/100 g)	6.49	4.13*
Mean soil moisture content (%)	76.00	79.00
pH	6.00	4.90

Soil moisture values are from measurements at depths of 10 and 30 cm
*significant at 5% level

Shah Alam forms part of the conurbation of the Klang Valley and has been identified as an area that is very susceptible to high levels of acidification (Anonymous 1995). Rainfall pH is between 4.5–5.0 (Philip & Rizal 1997). SO_2 and NO_2 values in the vicinity are between 3–8 ppb and 20–40 ppb respectively in 1995 (Anonymous 1996). On the other hand, no reports have been published on the air quality at Pangkor Laut but the rainfall pH is between 5.2–5.6 (Anonymous 1995).

Air quality assessment

The SO_2 and NO_x concentrations were monitored during the sampling days at these sites. Air was sampled at a point of 1.8 m above the street level. Each sampling lasted about 30 minutes and consisted of one round trip, approximately 3 km, along the compound of the tested sites. Three rounds were made between 8.00 and 11.30 a.m. during the physiological data collection days, throughout the period between January 1994 and December 1995. The data were averaged to represent measurements around the compound, providing 10 average values for each round. Sulphur dioxide and nitrogen oxides were measured using toxic gas detectors (Caselia TX1 1, UK).

Plant sampling and analysis

Fully expanded sun leaves from each tree were chosen for measurements of photosynthesis and stomatal conductance. These measurements were taken between 9.30 and 10.30 a.m. and were taken three days week^{-1} every fortnight, at photosynthetic photon flux density (PFD) of 700 μmol m^{-2} s^{-1} or more and 70% RH. The sampling time was predetermined from a diurnal curve. Light response curves were made in the initial month to determine the light saturation at both sites and apparent quantum yield for CO_2 fixation was obtained from the initial slope of the curves.

Photosynthetic rates, stomatal conductance and photon flux intensity were determined using an infrared gas analyser (ADC LCA 4, UK). The intercellular CO_2 concentration (C_i) and stomatal limitation to photosynthesis were calculated based on the equations developed by Farquhar and Sharkey (1982).

Leaf water potential was measured with Scholander pressure chamber (PMS, USA) technique. Predawn and midday measurements were taken once throughout the entire study; during the dry and wet seasons.

Six trees were chosen from each site for sampling and were monitored for a period of 24 months (January 1994–December 1995). Data for stomatal conductance, photosynthetic rate, leaf water potential and intercellular CO_2 concentrations were analysed using the analysis of variance and SAS package.

Results

Air quality

The average SO_2 and NO_x concentrations recorded at the study sites are presented in Table 2. These pollutants were seldom detectable at Pangkor Laut. Sporadic incidences took place in 1994, which coincided with the nation-wide hazy spells. On the other hand, the pollutant concentrations at Shah Alam exceeded the Malaysian Guidelines. The maximum values were noted during the hazy months in 1994. The prolonged haze in 1994 had also contributed to higher average values at these sites. The present short-term (1-hour average) Malaysian air quality standards for SO_2 and NO_x are 0.13 and 0.17 $\mu g\ g^{-1}$ respectively.

Based on these values, the air in Shah Alam, hereafter referred to as the polluted site, is considered polluted when compared with Pangkor Laut. The latter, hereafter referred to as control, is the reference for comparing the changes in plant parameters obtained since pollutant levels were lower and it is the natural habitat for the species.

Table 2 Minimum, maximum and average concentration of NO_x and SO_2 ($\mu g\ g^{-1}$) at the study sites

	Shah Alam		Pangkor Laut	
	SO_2	NO_x	SO_2	NO_x
Minimum	0.02	0.01	0.0	0.0
Maximum	0.40	0.40	0.01	0.01
Average	0.22	0.20	0.0	0.0

Readings were taken between 8.00 and 11.00 a.m. on sampling days.

Leaf water potential (ψ)

No significant differences were noted in leaf water potential values between the sites (Table 3). ψ was about - 0.50 and - 3.00 MPa for predawn and midday values respectively. In addition, there was no significant difference in the values between dry and wet seasons. These results suggested that the trees were not subjected to any water stress.

Light response of photosynthesis

Although, the light compensation point (l) for *P. pinnata* grown in polluted and control sites did not differ significantly, there was a decrease in CO_2 uptake (Figure 1). The leaves at the polluted site recorded an 11% decrease in the photosynthetic capacity at light saturation (1000 μmol m^{-2} s^{-1}), when compared with the control site (Table 4). The apparent quantum yield for CO_2 (ϕ_{CO_2}) in the polluted site was reduced by 37% compared with the control site (Table 4).

Table 3 Plant water potential (Ψ ± SE MPa)

Site	Wet season		Dry season	
	Predawn	Midday	Predawn	Midday
Control	- 0.52 ± 0.05	- 2.70 ± 0.16	- 0.61 ± 0.03	- 2.99 ± 0.08
Polluted	- 0.50 ± 0.10^{ns}	- 2.13 ± 0.28^{ns}	- 0.58 ± 0.07^{ns}	- 2.91 ± 0.05^{ns}

ns = not significant at 5% level
Each value is the mean of six replicates

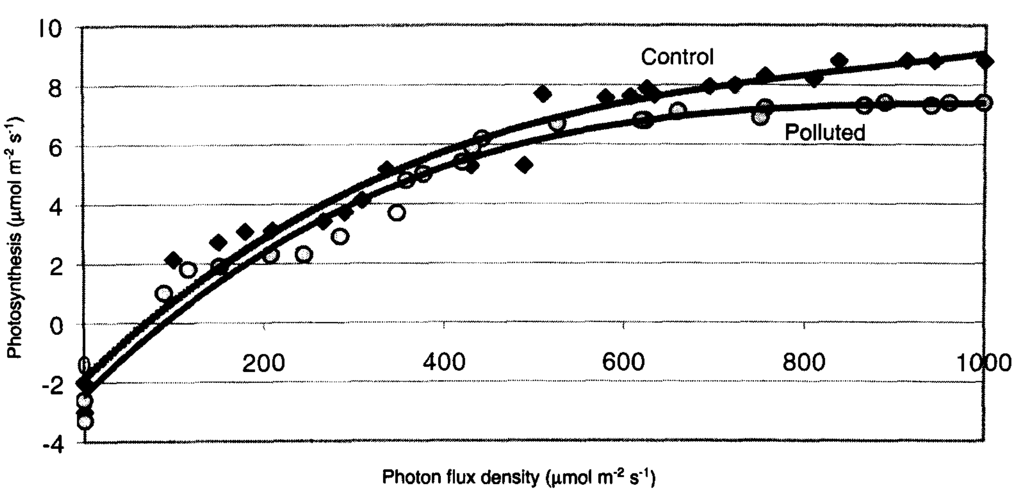

Figure 1 An example of light response of photosynthesis (Measurements were taken in the field at 28 °C and 67% RH)

Light saturated photosynthesis

Photosynthesis at light saturation in both polluted and control sites are shown in Figure 2. The monthly average photosynthetic rates (A) in the polluted site was 60% of that grown in the control site (Figure 2). As shown in Figure 2, the drop in stomatal conductance (g_s) followed the same trend as photosynthesis. The monthly average stomatal conductance was 70% of that grown in the control site (Figure 2).

Table 4 Estimated values of photosynthesis at light saturation (A_{max}, µmol m^{-2} s^{-1}), and apparent quantum yield for CO_2 (ϕ_{co_2}) fixation of light response curve of *Pongamia pinnata*

Site	A_{max}	ϕ_{co_2}
Control	8.3	0.035
Polluted	7.4*	0.022*

Values were derived from light response to photosynthesis curve.
*Significant at 1% level

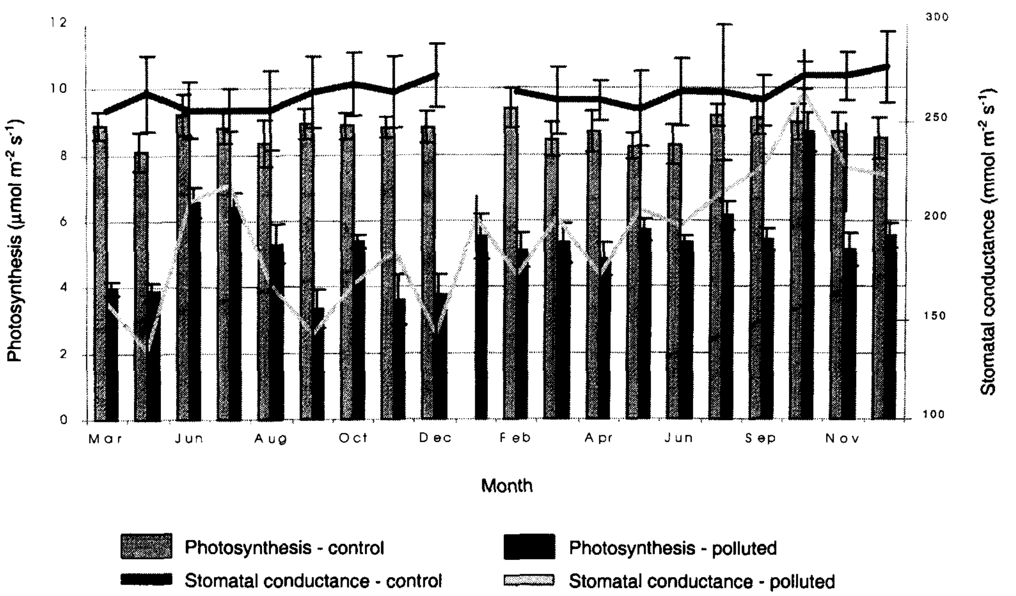

Figure 2 Mean monthly stomatal conductance and photosynthetic rates obtained from leaves of *Pongamia pinnata*
(Measurements were taken between 9.00 and 10.00 a.m.; temperature 28 °C and 67% RH. Values are mean of six replications; vertical bars are SE.)

Analyses of the results suggested that both g_s and *A* were dependent on rainfall prior to sampling and the SO_2 and NO_x levels in the atmosphere in the polluted site (Figures 3 and 4). A significant relationship ($p < 0.01$) existed between total amount of rainfall prior to sampling and g_s and *A*. *A* increased in the wetter sampling periods. With a rainfall of 80 mm and above, *A* increased to the values of the control site (Figure 3). On the other hand, *A* was uniform throughout the sampling period and no significant relationship was established between these parameters at the control site.

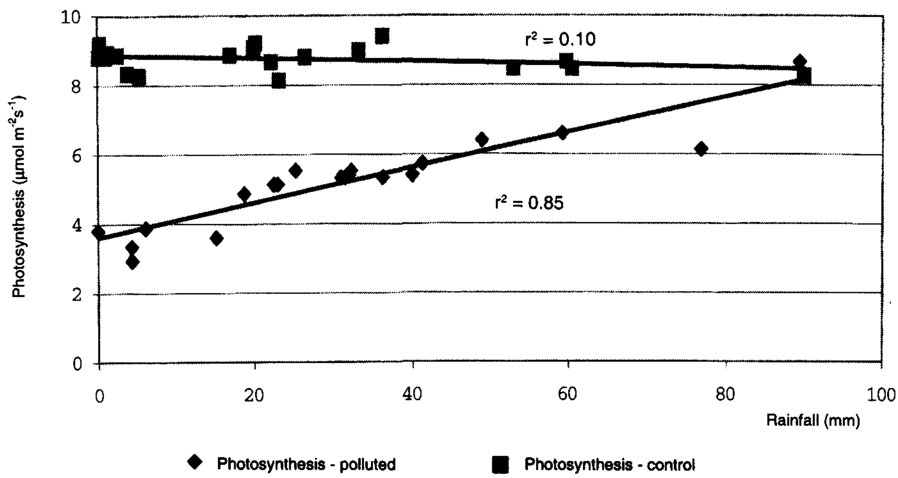

Figure 3 The relationship between photosynthetic rates and rainfall (The linear regression is significant at 0.01 level)

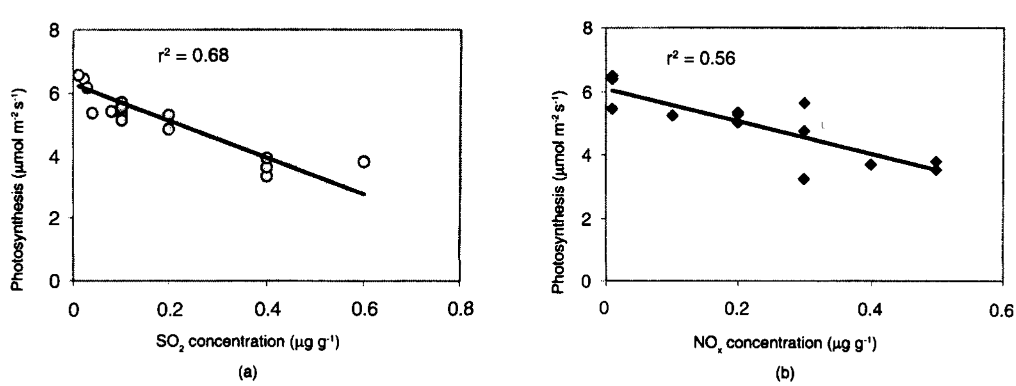

Figure 4 The relationship between photosynthesis and (a) SO_2 and (b) NO_x (The linear regression is significant at 0.01 level for both pollutants)

There was no significant difference in internal CO_2 concentration (C_i) at both sites, therefore suggesting that CO_2 was not the limiting factor for photosynthesis (Table 5). On the contrary, reduced photosynthetic activities caused accumulation of CO_2 in substomata cavities of leaves in the polluted site.

Table 5 Calculations of stomatal limitation to photosynthesis

	Control	Polluted
C_i (μmol mol^{-1})	295	302
C_a (μmol mol^{-1})	350	350
A = A at C_i (μmol mol^{-1})	8.36	6.47*
A_0 = A at C_a (μmol mol^{-1})	9.40	7.40*
Stomatal limitation (%)	11	13
Carboxylation efficiency	6.25	4.25*

*Significant at 5% level; C_a = atmospheric CO_2; A_0 = photosynthesis at atmospheric CO_2
All measurements were taken at 28 °C and 67% RH.

Analysis of the response of A/ C_i

The relationship between A and C_i differed for *P. pinnata* grown in polluted and control sites. Stomatal limitation decreased photosynthesis in both sites by about 12% from the potential assimilation rates at the ambient CO_2 concentration of 350 μmol mol^{-1} (Table 5). These values suggested that the reduced photosynthetic capacity was not due to the increase in stomatal resistance, but to the biochemical limitations to photosynthesis. This could be determined by the inferences from the A/C_i curves. It was noted that the carboxylation efficiency, inferred from the initial slope of the A/C_i curve, was significantly reduced in the polluted site compared with the control area (Table 5).

Discussion and conclusions

SO_2 and NO_x in Shah Alam, the polluted site, were higher than the minimum limit of the Malaysian Guidelines. SO_2 and NO_x were not detected at the control area. It ought to be highlighted that these values were the mean values for the experimental period and not the mean diurnal values. Darrall (1989) reported that SO_2 at concentrations between 200–400 ppb (0.2–0.4 μg g^{-1}) would significantly inhibit photosynthesis. The main source of SO_2 in Shah Alam is from the chimney of factories while the NO_x is from fuel burning equipment and vehicle exhaust. On the other hand, activities were kept to bare minimum at the control site.

Pongamia pinnata grown in Shah Alam produced a subsequent decrease in CO_2 uptake and stomatal conductance when compared with trees grown in Pangkor Laut. The reduction in A and g_s ranged between 20–40% of the control values and were comparable with those reported for other trees (Darrall 1989).

The current investigation revealed that photosynthesis at the polluted site was dependent on rainfall and the SO_2 and NO_x concentrations. Photosynthetic rates increased when sampling was done after a heavy downpour. Rainfall helps in dispersing air pollutants and cleaning the ambient air. When all other factors are equal, ambient levels of air pollutants tend to rise during the drier periods (Bower *et al.* 1991). The higher concentrations of pollutants are due to increased atmospheric stability and poorer dispersive capacity of the air.

Previous studies have shown that exposure of plants to SO_2 caused at least partial closure of stomata and report show that photosynthesis is the first to be affected (Carlson 1983, Furukawa & Totsuka 1983, Darrall 1989, Kumar & Dubey 1998). Nonetheless, there are also reports on direct effect of SO_2 on the guard cells (Black & Unsworth 1979). Stomata play a major role in regulating plant responses to atmospheric pollutants (Mansfield 1973). The capacity of plants to assimilate CO_2 is controlled by stomatal conductance and by internal biochemical and physiological processes (Temple 1986). Both A and g_s are coordinated in a manner that optimises daily assimilation rates (Farquhar & Sharkey 1982).

Results from the current investigation are in agreement with the argument that photosynthesis pathway was first impaired and stomatal closure was secondary. The decrease in the initial slope of A/C_i curve is dependent on the efficiency of primary carboxylase (Farquhar *et al.* 1980). The loss of carboxylation efficiency has contributed to the decline in A. The decrease in A_{max}, on the other hand, suggested a decrease in the RUBP regeneration. Thus, the mechanism of stomatal closure is predominantly secondary to the decrease in carboxylation. These observations are in line with the suggestion by Carlson (1983) and Furukawa and Totsuka (1983).

As highlighted earlier, these pollutants could be the cause of regular leaf fall observed in the polluted site. The depressed biochemical activities could have affected the health of the leaf and thus caused its shedding. Leaves exposed to SO_2 appeared chlorotic and tend to senescence rapidly (Whitmore *et al.* 1985). The chloroplast is a sensitive target for primary damage by SO_2 or its products in aqueous solution. Damage includes chlorophyll destruction and inhibition of photosystem II (Rennenberg & Polle 1994). In this case, chlorotic leaves were noted in the polluted site. Hence, more work needs to be done on the chlorophyll content, its functions and the biochemical pathway to fully understand the shedding behaviour.

In conclusion, SO_2 and NO_x reduced the CO_2 uptake as a result of reduced carboxylation efficiency. Changes in stomatal conductance have only a smaller quantitative effect on the rate of CO_2 uptake or it occurred later.

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