## การใช้แสงฟลูออเรสเซนซ์จากคลอโรฟิลล์ของไลเคนบ่งบอกคุณภาพอากาศของสวนสาธารณะในกรุงเทพมหานคร

# USING CHLOROPHYLL FLUORESCENCE OF LICHEN TO EVALUATE AIR QUALITY OF PUBLIC PARKS IN BANGKOK

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**บทคัดย่อ:** กรงเทพมหานครมีประชากรหนาแน่นและมีปัณหามลภาวะทางอากาศจากรถขนต์และอุตสาหกรรม การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อใช้ไลเคนประเมินความแตกต่างของคุณภาพอากาศในสวนสาธารณะใน กรุงเทพฯ โดยการวัดแสงฟลูออเรสเซนซ์จากคลอโรฟิลล์ของไลเคน Pyxine cocoes ซึ่งเติบโตอย่ใน ้สวนสาธารณะ 5 แห่ง โคยเก็บตัวอย่างไลเคน 5 แทลลัส เพื่อนำมาวัคค่าแสงฟลูออเรสเซนซ์จากคลอโรฟีลล์ ใน ้ห้องปฏิบัติการไลเคน มหาวิทยาลัยรามคำแหง โดยการปรับสภาวะระหว่างการวัดให้คงที่คือ ความชื้นอิ่มตัว อุณหภูมิ 25 ± 2  $^{\circ}$ C แสง 500  $\mu$ mol m $^{-2}$ s $^{-1}$  ค่าที่ใช้ในการประเมินแสงฟลูออเรสเซนซ์จากคลอโรฟิลล์ได้แก่ Fo, Fm-Ft/Ft พบว่า ไลเคนจากสวนสาธารณะที่อยู่ใจกลางกรุงเทพฯ มีค่า Fv/Fm, Fm-Fv/Fm, **ΦPSII** และ Ft/Ft และ ΦPSII ต่ำกว่าไลเกนจากสวนสาธารณะที่อย่ชานเมือง โดยอาจเกิดจากการได้รับมลพิษในรปของ ใน ใตรท และแอมโมเนียจากรถยนต์ ซึ่งมลพิษเหล่านี้พบความเข้มข้นสูงในบริเวณเมือง ส่วนไลเคนที่เติบโตใน ้สวนสาธารณะที่อยู่ชานเมืองมีก่าแสงฟรูออเรสเซนต์ ฯ สูงกว่า เนื่องจากสาร  $\mathrm{NO}_3^-$  และ  $\mathrm{NH_4^+}$  ในกวามเข้มข้นที่ เหมาะสม ถูกนำไปใช้เป็นสารอาหารเช่นเคียวกับปุ๋ย ช่วยเพิ่มประสิทธิภาพในการส่งถ่ายอิเล็กตรอนของ PSII และ ้การเติบโต แต่เมื่อห่างจากเมืองมากเช่น อทยานแห่งชาติเขาใหญ่ ซึ่งเป็นพื้นที่ควบคม ค่าดังกล่าวลดลงมาก เนื่องจากไลเคนถูกจำกัดด้วยสารอาหาร สามารถเรียงลำดับสวนสาธารณะที่มีคุณภาพอากาศของสวนสาธารณะจากดี ที่สุดและลดลงได้ดังนี้ สวนธนบุรีรมย์ สวนพระนกร สวนหลวง ร.ธ สวนเสรีไทย และสวนลุมพินี ตามลำดับ

**Abstract:** Bangkok has very dense population with problem of air pollution caused by heavy traffic and industries. The objective of this study is to evaluate air quality of five public parks by measuring chlorophyll fluorescence of the lichen *Pyxine cocoes*, which commonly grows in five public parks in Bangkok. Five thalli were taken from each park and chlorophyll fluorescences were measured under laboratory condition at Ramkhamhaeng University. The condition during the measurement in the laboratory was kept constant at saturate humidity, temperature  $25\pm2$  °C and light intensity 500 µmol m<sup>-2</sup>s<sup>-1</sup>. Chlorophyll fluorescence parameter included Fo, Fv/Fm,  $\Phi$ PSII and (Fm-Ft)/Ft. The result showed that lichens growing in the inner city had the lowest values of Fv/Fm and  $\Phi$ PSII, which possibly caused by toxic effect of nitrate and ammonia from automobile exhaust. These nitrogenous air pollutants had higher concentrations in the city than in the suburbs and rural areas. Lichens from the outer city parks had higher values of these parameters, indicating that electron transports of PSII were enhanced by NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> through the process known as fertilization effect. The lowest value of chlorophyll fluorescence parameter was measured from lichen collected from KYNP, which revealed that it is nutrient limited. Air quality among the five public parks in Bangkok can be ranked from the best to the poorest as follow, Thonburirom, Pranakorn, Rama 9, Seri Thai and Lumpini.

Introduction: Public parks in Bangkok are regarded as lung of the urban people. They located in various part of the city, which may have different air quality due to density of traffics and

distances from industries. Pollutants in the city with heavy traffic mainly composed of nitrogen oxide, carbon monoxide, hydrocarbon, ozone and lesser amount of sulfur dioxide and heavy metals. Lichens are epiphyte, which depend on water and nutrients from the atmosphere. Therefore, they are sensitive to air pollution, and usually disappear from polluted areas. However, some lichens survive pollutants, and grow well in the cities. These species included e.g. Lecanora conizaeoides, L. dispersa and Pyxine cocoes [1,2]. Lichens grow in the urban areas accumulated pollutants and capable of metabolize some small quantities as sources of nutrients e.g. nitrite, nitrate, ammonia, and even sulfur dioxide from the atmosphere [3,4]. However, accumulation of toxic substances beyond their metabolic capabilities can alter biochemical and physiological functions which lead to morphological changes and death. Chlorophyll fluorescence of lichens has been widely used recently to measure state of lichen caused by environmental stress including air pollution [5]. The objective of this study was to evaluate air quality in public parks in Bangkok by measuring chlorophyll fluorescence of lichens that grow in these parks. Our hypothesis is, air quality of public parks in Bangkok is different, and can be evaluated by measuring chlorophyll fluorescence of lichens inhabited these parks.

#### Materials and methods

**Lichens and public parks:** The lichen *Pyxine cocoes* were collected from five public parks in Bangkok namely, Thonburirom, Rama 9, Seri Thai, Pranakorn and Lumpini during 10 January 2008. The same species of lichen were also collected from Khao Yai National Park (KYNP) to use as control materials. Five thalli were collected from each location from host trees with circumference over 50 cm. at levels 50-200 cm. above ground. The samples were kept at the laboratory at Ramkhamhaeng University under room temperature, ambient condition of humidity, light and dark period. Chlorophyll fluorescences were measured within 3 days by using MINI PAM [6]. The samples under went dark adapted for 12 hour before the measurement. The condition during the measurement were 25 °C, over 95 % RH and 500  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> actinic light intensity. The measurements from each location. After means and standard derivation were calculated, the values of the fluorescence parameters were normalized by dividing with values of the control site. This is just to make comparison simple.

**Principle and method of chlorophyll fluorescence:** Light energy absorbed by chlorophyll is allocated by three major routes: 1) passing to PSII for photochemistry in photosynthesis 2) dissipate as heat 3) loss in longer wavelength as chlorophyll fluorescence. The three processes occur in completion, such that any increased in one will result in decrease of the other two. After dark adapted, the thalli were exposed to weak light of 0.5 µmol m<sup>-2</sup>s<sup>-1</sup>, of which background chlorophyll fluorescence (Fo) can be determined. Thereafter, a saturation pulse of 5,000 µmol m<sup>-2</sup>s<sup>-1</sup> for 0.8 second was applied. This condition allows chlorophyll fluorescence to reach its peak (Fm), and maximum capacity of PSII (Fv/Fm) can be calculated from (Fm-Fo)/Fm. The actinic light of 500 µmol m<sup>-2</sup>s<sup>-1</sup> was then applied for 1 minutes until steady state chlorophyll fluorescence (Ft) was reached. By applying another saturating pulse, chlorophyll fluorescence peak again (Fm'), but lower than before (Fm) because a portion of energy was lost as heat. These values were used to determine:

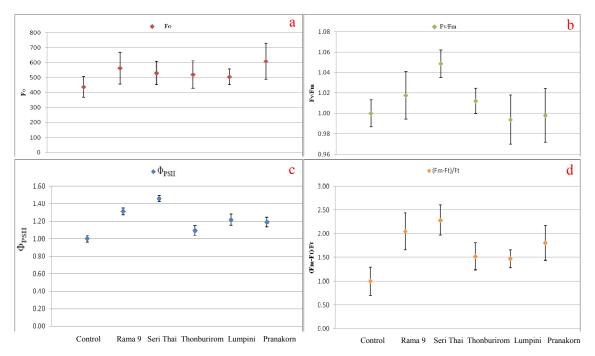
$\Phi_{\rm II}$ or (Fm'-Ft)/Fm'	Quantum yield of electron transport though PSII
(Fm-Ft)/Ft	Vitality index, a measure of stress effects in PSII

#### Result discussion and conclusion

**Responses of lichens to air pollution:** Chlorophyll fluorescence parameters of the lichen *P. cocoes* grew in the five public parks in Bangkok varied and different from those inhabited KYNP (Table 1 and Figure 1). It reveals that these lichens were exposed to different levels of stress factors consisting of e.g. dessication, nutrient deficit, nutrient toxicity, as well as pollutants. Concentration of nitrate (NO<sub>3</sub><sup>-</sup>), ammonia (NH<sub>4</sub><sup>+</sup>) and sulfate (SO<sub>4</sub><sup>2-</sup>) in Bangkok were 5-3 times higher than those measured from rural (Kanchanaburi) and other cities (Samutprakarn, Pratumtani, Nakornrachasima and Chiang Mai) [7]. Nitrate and ammonia are toxics, however, lichens can utilize them as nutrient through enzymatic metabolism. In appropriate quantity they enhance physiological performance and growth, the process known as fertilization effect [3,4]. However, excess quantities of these compounds become toxic because limitation of metabolic enzymes for detoxification. Sulfate plays similar role, as plant nutrient at lower and as toxic substance when present in high concentration.

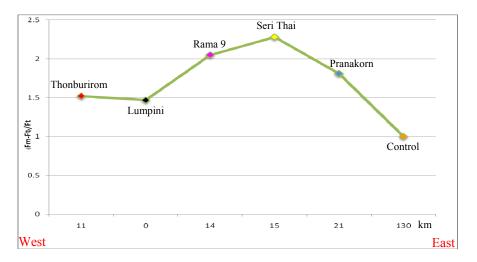
**Table 1.** Means and SD of Fo with normalized values of Fo, Fv/Fm,  $\Phi$ PSII and (Fm-Ft)/Ft from lichens inhabited five public parks in Bangkok and Khao Yai National Park.

			Public parks	Public parks			
Parameters	Control	Seri Thai	Rama 9	Lumpini	Pranakorn	Thonburirom	
Means ± SD							
Fo	439±70.2	531±78	564±105.8	506±51.6	610±120.5	521±90.7	
Normalized values							
Fo	1.00	1.21	1.28	1.15	1.38	1.19	
Fv/Fm	$1.00\pm0.01$	$1.05 \pm 0.01$	$1.02 \pm 0.02$	$0.99 \pm 0.02$	$1.00{\pm}0.02$	$1.01{\pm}0.01$	
ΦPSII	$1.00\pm0.03$	1.46±0.03	1.31±0.03	$1.22 \pm 0.06$	1.19±0.05	$1.09{\pm}0.05$	
(Fm-Ft)/Ft	$1.00{\pm}0.02$	2.28±0.31	2.05±0.39	$1.47 \pm 0.18$	1.81±0.36	$1.52 \pm 0.28$	



**Figure 1.** Normalized values of chlorophyll fluorescence parameters Fo (a), Fv/Fm (b),  $\Phi_{II}$  (c) and (Fm Ft)/Ft (d) of *P. cocoes* from five public parks in Bangkok and Khao Yai National Park. Lichen in Bangkok had  $\Phi$ PSII, and (Fm-Ft)/Ft values higher than KYNP, because of fertilization effect.

Variation of chlorophyll fluorescence parameters measured from lichens in Bangkok and KYNP can be interpreted, in part, by law of tolerance based on fertilization and toxic effects of the pollutants. It means that low concentration of these pollutants slightly enhance electron transport through PSII [5]. As the concentration of the nutrient increase until it reach the optimum level, electron transport also increase to the highest value. Concentration over the optimum level, nitrate and ammonia become toxic and cause decline of electron transport to PSII [8]. Therefore too low or too high concentrations of these nitrogen pollutants result in inefficient electron transport trough PSII.



**Figure 2.** Vitality index (Fm-Ft)/Ft of lichens from five public parks, from west to east, in Bangkok and Khao Yai National Park. This value indicates stress effects in PSII or changes in photosynthetic apparatus.

**Evaluating air quality of public parks in Bangkok:** Chlorophyll fluorescence parameter, especially vitality index, along with biodiversity survey [5] seems to be the effective means to evaluate air quality of public parks in Bangkok. Figure 2. shows location of public parks in Bangkok from east to west related to vitality index (Fm-Ft)/Ft, of which air qualities can be evaluated.

Lumpini park located at the center of the city had the lowest value of vitality index. This park had only four species of lichens. It is reasonable to assume that Lumpini had the worst air quality caused by nitrogen deposition. Other factors probably involved e.g. hydrocarbon, heavy metal and dessication, which remain unresolved. Away to the west is Thonburirom park, which had the second lowest vitality index among the park in Bangkok. It can have either too low or too high nitrogenous pollutants. However, as many as twelve species of lichens inhabited this park, it should be regarded as receiving low nitrogen deposition, which means the best air quality. Along the eastern part of the city, Pranakorn and Rama 9 parks should be considered as having the second and the third best air quality due to higher values of vitality index, with six and nine species of lichens found. Seri Thai should be ranked the four better air quality, with the highest value of vitality index and seven species of lichens presented. It implied that nutrient deposition to this park was benefit to the lichens. KYNP had the lowest value of (Fm-Ft)/Ft among all observations, which indicated its best air quality, despite lichens were limited by nutrient especially nitrogen.

In conclusion, chlorophyll fluorescence is an effective parameter for evaluating physical performance of lichens, and consequently air quality of their habitats. The interpretation can be improved, in the future, by developing a relationship of nutrients and pollutants accumulate in thalli with chlorophyll fluorescence parameters. This information would enhance our

understanding on threshold of pollutants that affect health, of which policy and measure could be developed to mitigate detrimental effects of air pollution.

### **References:**

- 1. J.R. Laundon., Lichen Flora of London., 1967, 277-327.
- 2. T. Kulapirak., Master's thesis. Chiang Mai University, Chiang Mai. 2006.
- 3. S. Parnmen and K. Boonpragob., 29<sup>st</sup> Congress on Science and Technology of Thailand. 2005.
- 4. L. Frati, E. Caprasecca, S. Santoni, C. Gaggi, A. Guttova, S. Gaudino, A. Pati, S. Rosamilia, S.A. Pirintsos, S. Loppi., *Environmental Pollution.*, 2005, **142**, 58-64.
- 5. M. Kummerova, M. Bartak, J. Dubova, J. Triska, E. Zubrova, and S. Zezulka,

Ecotoxicology., 2005, 15, 121-131.

- 6. Heinz Waltz GmbH., Photosynthesis Yield Analyzer MINI-PAM., Germany., 1999.
- 7. Network Center for EANET., Acid Deposition Monitoring Network in East Asia., 2007.
- 8. K. Boonpragob and T.H. Nash III., Environ. Exp. Bot., 1991, 31, 229-238.

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