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Accumulation of inorganic pollutants and photosynthetic responses of transplanted lichens at distances away from an industrial complex

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Abstract. Chemical composition and physiological responses of lichens are widely used as indicators for assessing environmental alteration in several land uses. Therefore, the objective of this study was to utilize ion contents and photosynthetic parameters in the lichen *Parmotrema tinctorum* to evaluate air quality in industrial, agricultural and natural areas in Thailand. The lichen samples were collected in a relatively unpolluted area, and then transplanted at one natural site in the Khao Yai National Park, one agricultural site and three industrial sites in Map Ta Phut. During the exposure period of 8 months (October 2013 to June 2014), the transplanted lichens were periodically collected 4 times for analysis of ion contents including Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-}, as well as for measurement of photosynthetic parameters including net photosynthesis, chlorophyll fluorescence (Fv/Fm), total chlorophyll (a+b) content and the degradation of chlorophyll a, which was estimated by OD435/OD415 ratio. The mean concentrations of all ions had increasing trends at sites closer to the industry. The monitoring site that was located downwind of the industrial core center for a longer period of time had higher ion concentrations. All photosynthetic parameters were conspicuously lower at the closest sites to the industry, and had negative correlations with the accumulated ion contents. This study clearly confirmed that lichen can be used as a bioaccumulator of inorganic pollutants and a bioindicator of atmospheric purity, which is appropriate for developing countries where air quality monitoring instruments are insufficient.

Keywords: air quality, chlorophyll fluorescence, Map Ta Phut, Net photosynthesis, *Parmotrema tinctorum*

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INTRODUCTION

A major anthropogenic source of inorganic ions such as Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-} is fossil fuel combustion. These substances are toxic to respiratory and nervous systems in humans (WHO, 2000; Kampa & Castanas, 2008). As such, it is necessary to assess their deposition in order to evaluate their impacts on human health and environment.

Lichens acquire water and nutrient, including pollutants directly from the atmosphere. They can accumulate elements far above their need (Garty & Garty-Spitz, 2015), and several substances cause adverse effects on their physiological process (Paoli *et al.*, 2016; Sujetovienė & Galinytė, 2016). Thus, the chemical composition and physiological responses of lichens reflect air quality/ purity of their habitat (Yemets *et al.*, 2014; Paoli *et al.*, 2015; Mateos & González, 2016).

Biomonitoring of air pollution with lichens is widely recognized, especially in Europe and America (Brunialti & Frati, 2014; Will-Wolf *et al.*, 2015). This technique is cheaper and easier comparing with using air monitoring equipment because it spends fewer budgets and does not need electricity and maintenance for operation. Moreover, lichens are able to detect several pollutants even they are present at very low concentrations in the environment (Garty & Garty-Spitz, 2015; Protano *et al.*, 2015; Boonpeng *et al.*, 2017).

Therefore, the main target of this study was to use accumulated inorganic ions and changes in photosynthetic parameters of the transplanted lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale to evaluate air quality in industrial, agricultural and natural areas in Thailand.

MATERIALS AND METHODS

Monitoring sites and lichen transplantation

This study was carried out in natural, agricultural and industrial areas, encompassing a total of five monitoring sites (Table 1). These sites were selected based on the distance and direction from the Map Ta Phut industrial core center.

TABLE 1. Description of five monitoring sites.

Site	Location	Distance–Direction*	Remark
1	Khao Yai National Park (14°25'12" N 101°22'23" E)	193 km–north	A relatively clean air site used as control
2	Thep Nimit Temple (12°51'23" N 101°25'09" E)	35 km–northeast	An agricultural/ rural area near Wang Chan District, Rayong province
3	Khao Phai School (12°43'12" N 101°12'17" E)	8 km–northeast	A peri-industrial site in the Map Ta Phut industrial area
4	Sophonwanaram Temple (12°42'32" N 101°09'50" E)	3 km–northeast	An industrial site in the Map Ta Phut industrial area
5	Bannongfab School (12°41'08" N 101°06'58" E)	3 km–southwest	An industrial site in the Map Ta Phut industrial area

* Approximate distance and direction from the Map Ta Phut industrial center (12°41'28" N 101°08'25" E).

The epiphytic foliose lichen *P. tinctorum* was chosen. This species was recently confirmed as an effective biomonitor of atmospheric deposition (Käffer *et al.*, 2012; Dohi *et al.*, 2015). Only peripheral parts of the thalli, about 2–3 cm from the lobe tips, were selected. Each thallus fragment of approximately 10 cm^2 was fixed on a frame of polyethylene netting (2 × 2 mm mesh size) using nylon string. The transplanted materials were placed at the control site for approximately 4 months to allow the physiological adaptation and homogenization of the samples. The lichen material was then transferred to the five monitoring sites on October 18, 2013, by fixing on tree branches, approximately 3–4 m above ground to avoid soil contamination. The transplanted period lasted for 8 months (October 2013 – June 2014), and during the exposure period they were collected 4 times: December 8, 2013, January 27, 2014, March 24, 2014 and June 16, 2014, for analysis of accumulated pollutants and measurement of photosynthetic parameters.

Analysis of inorganic ions

The analytical method followed the procedure by Jhumpasri *et al.* (2016). Approximately 200 mg of the lichen material was extracted by an H_2O treatment in an ultrasonic bath (950DAE, Crest Ultrasonics, USA) for 10 min. Four inorganic anions including chloride (Cl^-), nitrate (NO_3^-), sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) were determined and quantified with a DX100 ion chromatograph (IC, Dionex, Sunnyvale, CA, USA) using an IonPac AS12A analytical column, an IonPac AG12A guard column, an ASRS 300 (4 mm) as a suppressor and a mixture of 2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ as eluent. The analytical quality was checked by precision and spike recovery. The results of precision (n=7) expressed as percent relative standard deviation (%RSD) were less than 7%, and the recoveries (n=7) of all ions ranged between 90–107%. Three replicates were determined from each monitoring site at each collection time and results were expressed on dry weight basis (µg/g dw).

Net photosynthetic rate

Each lichen sample was moistened by spraying distilled water and incubated under photosynthetic photon flux density (PPFD) *ca*. 100 µmol m^{-2} s^{-1} using light emitting diode (LED) lamp, in a controlled temperature room of about 26±2 °C for 2 h. The measurement of net photosynthesis (NP) was carried out with a portable LI-6400 infrared gas analyzer (IRGA, Li-Cor, Lincoln, NE, USA) attached to a conifer chamber, operating in an opened system. Conditions used for the CO_2 gas exchange measurement were: PPFD = 350 µmol m^{-2} s^{-1} generating by an external LED lamp (50W), thallus water content = 80–100% air dry weight, temperature = 26±2 °C, air flow rate = 500 µmol s^{-1}, and at ambient CO_2 (300–400 ppm) (Boonpeng, 2011). Four replicates were measured from each monitoring site at each collection time, and results were expressed on area basis (µmol CO_2 m^{-2} s^{-1}).

Chlorophyll fluorescence

Each sample was moistened by spraying distilled water and placed in dark condition for 1 h, at 26±2 °C. Chlorophyll fluorescence measurements were carried out on well-wet samples, with a pulse amplitude modulated fluorometer MINI-PAM (Heinz Walz GmbH, Effeltrich, Germany), applying a saturating light pulse of *ca*. 8000 µmol m^{-2} s^{-1} for 0.8 s. The values of Fv/Fm = (Fm–F_0)/Fm, which indicates the maximum possible efficiency of photosystem II (PSII) of the lichens (Jensen & Kricke, 2002) were measured from twelve replicates at each site and collection time.

Chlorophyll content and degradation

Samples of 50 mg (air-dry weight) were washed with approximately 2 ml of $CaCO_3$-saturated 100% acetone for *ca*. 1 min, six times. The washing process removed lichen substances, which cause chlorophyll degradation during the process of extraction (Barnes *et al.*, 1992). After acetone evaporated from the samples, 5 ml of dimethyl sulfoxide (DMSO) containing 2.5 mg/ml polyvinylpolypyrrolidone (PVP) was added, and subsequently incubated in a hot air oven at 65 °C for 45 min in the darkness (Barnes *et al.*, 1992; Boonpragob, 2002). The extracts were in dark and allowed to cool in ambient temperature, and then dilute 1:1 by adding another 5 ml DMSO. The optical density of the solution was measured at wavelengths 415, 435, 648 and 665 nm with a spectrophotometer (GENESYS 10S UV-Vis spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). Total chlorophyll (a+b) content (chl a+b) was calculated using the equations given by Barnes *et al.* (1992). As well as, the chlorophyll degradation was estimated by using the ratio of chlorophyll a/phaeophytin a (OD435/OD415), as suggested by Ronen & Galun (1984). Four replicates were extracted from each site and collection time, and the total chlorophyll content was expressed on dry weight basis (mg/g dw).

Statistical analysis

Statistical differences of the ion contents and the photosynthetic parameters among the monitoring sites were analyzed using 95% confidence interval. Relationships between the ions and the photosynthetic parameters, and distance from the industrial center were tested using Pearson's correlation (*r*). All statistical analyses were performed using SPSS software (Version 22, IBM Corp, Armonk, NY, USA) and SigmaStat 3.5 (Systat Software, San Jose, CA, USA).

RESULTS AND DISCUSSION

Accumulation of ions in lichens

Content of all ions in the transplanted lichen *P. tinctorum* at the monitoring sites during the study period of 8 months was shown in Table 2. The mean concentrations of all ions were evidently higher at the industrial sites than the control and rural sites. The sites that were located closure to the industrial center showed higher contents than the farther ones and Pearson's correlation coefficients (*r*) revealed that the ions tended to increase with decreasing distance from the Map Ta Phut industry (Table 3). The highest mean concentrations of Cl^-, NO_3^- and SO_4^{2-} were observed at site 4, which was only 3 km northeast of the industrial center . Similar findings were also reported elsewhere. Boonpragob *et al.* (1989) revealed that amounts of Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-} in the lichen *Ramalina menziesii* were significantly higher at a polluted site than a control site in California, USA. Likewise, Garty *et al.* (2001b) discovered that concentrations of Cl^-, NO_3^- and SO_4^{2-} in the lichen *R. lacera* transplanted in the industrial area at Haifa Bay, Israel, were substantially higher than those at an unpolluted site. In addition, Naeth & Wilkinson (2008) found that SO_4^{2-} in lichens at site close to an industrial area (diamond mine) showed higher content than that at sites further away. Furthermore, Mateos & González (2016) reported that sulfur content in the lichen *Usnea amblyoclada* exposed in an industrial area (metallurgical and metal-mechanic industries) were significantly higher than that at a control location.

Ion contents in the lichens also differed depending on the direction from the industrial core center. Site 4 and 5 were located at about 3 km away from the industrial center, but on the opposite direction, northeast and southwest. Concentrations of all ions were higher at site 4 than site 5. This could be the consequence of the path of prevailing wind. It blew from northeast to southwest in the first 102 days (October 18, 2013 to January 27, 2014) of the study period, and *vice versa* in the remaining 140 days (January 28, to June 16, 2014), meaning that site 4 was situated downwind of the industry for a longer period of time than site 5. Thus, the wind picked up pollutants from the industry and deposited at site 4 larger than those at site 5.

These inorganic ions are mainly originated from anthropogenic activity. Chloride possibly discharged from chemical related factories. Moreover, it probably associated with polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). These persistent organic pollutants (POPs) are unintentional by-products emitted from combustion in many industrial chemical processes (Augusto *et al.*, 2015; Protano *et al.*, 2015). Nitrate and sulfate mainly originated from fossil fuel combustion (Vallero, 2008). Lastly, phosphate might be released from the combustion of oil and coal, and phosphate-related factories (Tipping *et al.*, 2014).

More importantly, the quantities of NO_3^- and SO_4^{2-} in lichens showed similar trends with NO_2 and SO_2 in the air, which were measured at the air quality monitoring stations by the Air Quality and Noise Management Bureau, Pollution Control Department (PCD), Thailand (Fig. 1). It strengthened the evidences that lichens can be efficiently used as biomonitors of atmospheric pollutants.

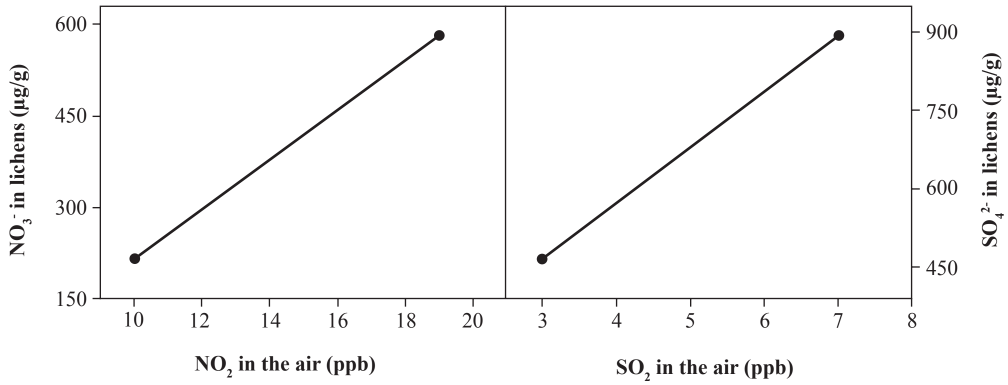

FIGURE 1. Relationship between the concentrations of nitrogen and sulfur compounds in the transplanted lichens (NO_3^- and SO_4^{2-}) and the air (NO_2 and SO_2) during the study period.

Photosynthetic parameter responses

All photosynthetic parameters of the transplanted lichens were conspicuously lower at the closet industrial sites from the industrial center (Table 2), and they tended to increase with increasing distance from the industry (Table 3). Photosynthetic rates and total chlorophyll contents in lichens were widely used as indicators of environmental stress in several land uses (Zambrano & Nash III, 2000; Häffner *et al.*, 2001; Majumder *et al.*, 2013). For example, Garty *et al.* (2001a) reported that the photosynthetic rate and chl a+b content of transplanted lichens were significantly lower at industrial sites than those from rural sites. The Fv/Fm values of the transplanted lichens at the closest sites to the Map Ta Phut industry (sites 4 and 5) were evidently lower than the control site. This parameter is a useful tool for evaluating the effects of air pollution on living organisms (Fernández-Salegui *et al.*, 2006; Tretiach *et al.*, 2007; Paoli *et al.*, 2014). A recent study by Sujetovienė & Galinytė (2016) discovered that the Fv/Fm values of the lichen *Evernia prunastri* transplanted in urban and residential areas were lower than those at suburban and control sites. Chlorophyll a degradation, which was estimated by OD435/OD415 ratio (Ronen & Galun, 1984), at site 4 it showed the lowest value. This parameter frequently uses as an early sign of environmental stress in study areas (Lucadamo *et al.*, 2015; Paoli *et al.*, 2015).

All of the investigated inorganic ions showed adverse effects on the photosynthetic parameters, especially nitrate and sulfate had significantly negative correlations with OD435/OD415 ratio (Table 3), indicating that the amounts of these ions could be toxic to the lichen physiology. This result was similar with the observations of Boonpragob & Nash III (1991) and Garty *et al.* (2001b).

Table 2. Means, lower and upper bounds of inorganic pollutants (n=12), photosynthetic parameters including net photosynthesis (NP), chlorophyll a+b (chl a+b), chlorophyll degradation (OD435/OD415) (n=16) and chlorophyll fluorescence parameter (Fv/Fm) (n=48), calculated using 95% confidence interval.

Pollutant/ Parameter	**Site 1** control-193 km-north			**Site 2** rural-35 km-northeast			**Site 3** peri-industrial-8 km-northeast			**Site 4** industrial-3 km-northeast			**Site 5** industrial-3 km-southwest		
Pollutant (µg/g)	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper
Cl^-	103	**131**	159	129	**169**	210	175	**294**	413	191	**298**	405	160	**251**	342
NO_3^-	126	**159**	192	132	**174**	216	143	**193**	243	134	**454**	773	152	**217**	282
SO_4^{2-}	279	**381**	482	306	**454**	602	458	**650**	842	645	**890**	1135	361	**466**	571
PO_4^{3-}	611	**723**	844	530	**768**	1007	857	**1082**	1307	760	**1049**	1337	771	**955**	1138
Photosynthetic parameter															
NP (µmol CO_2 m^{-2} s^{-1})	2.25	**2.62**	2.99	2.02	**2.48**	2.94	2.34	**2.80**	3.25	1.29	**1.96**	2.62	1.63	**1.97**	2.31
Fv/Fm	0.63	**0.65**	0.67	0.55	**0.58**	0.61	0.62	**0.63**	0.64	0.46	**0.50**	0.54	0.47	**0.49**	0.52
Chl a+b (mg/g)	0.85	**0.99**	1.23	0.94	**1.10**	1.26	0.87	**1.04**	1.22	0.66	**0.88**	1.10	0.74	**0.86**	0.99
OD435/OD415	1.22	**1.25**	1.29	1.21	**1.25**	1.29	1.20	**1.24**	1.28	1.09	**1.17**	1.26	1.20	**1.24**	1.28

TABLE 3. Pearson's correlation coefficients (*r*) (n=5) among the photosynthetic parameters including net photosynthesis (NP), chlorophyll fluorescence parameter (Fv/Fm), chlorophyll a+b (chl a+b) and chlorophyll degradation (OD435/OD415), ion contents and distance from the Map Ta Phut industrial center, * $p<0.05$, *** $p<0.001$.

	NP	Fv/Fm	Chl a+b	OD435/OD415	Cl^-	NO_3^-	SO_4^{2-}	PO_4^{3-}
Fv/Fm	**0.96***							
Chl a+b	0.83	0.73						
OD435/OD415	0.64	0.62	0.60					
Cl^-	-0.53	-0.66	-0.58	-0.76				
NO_3^-	-0.65	-0.62	-0.56	**-1.00*****	0.70			
SO_4^{2-}	-0.40	-0.46	-0.39	**-0.93***	0.85	**0.91***		
PO_4^{3-}	-0.23	-0.39	-0.37	-0.62	**0.95***	054	0.80	
Distance	0.43	0.67	0.20	0.44	-0.81	-0.41	-0.57	-0.75

CONCLUSION

The monitoring site that was adjacent to the Map Ta Phut industrial core center evidently exhibited worse air quality than those faraway, especially the site that was located downwind of the industry for a longer period of time. This study clearly affirmed that lichen can be used as a bioaccumulator of inorganic pollutants and a bioindicator of atmospheric purity. It is appropriate for developing countries where air quality monitoring instruments are insufficient.

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