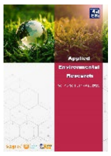
App. Envi. Res. 46(1) (2024): 002

Applied Environmental Research

- RESEARCH FOR SUSTAINABLE PLANET -



Research Article

Photosynthesis-related Parameters in *Leucobryum aduncum* Moss Bags as Bioindicators of Environmental Stress Caused by Road Traffic

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Abstract

The physiological response in moss samples is often used as an early warning signal of environmental stress caused by air pollution. This study aimed to test the efficiency of photosynthesis-related parameters in *Leucobryum aduncum* moss bags as bioindicators of environmental stress related to road traffic. The moss samples were collected and prepared in a relatively unpolluted area and then transplanted at 7 sites in forested, rural, and urban areas. The moss samples were picked up after being exposed for 1 and 3 months for physiological measurements. The results showed that the average values of all physiological parameters were highest in the forested area and lowest in the urban area. All parameters at most roadside (R site) sites were lower or significantly lower than those at the site away from the road (A site). This was particularly true for the 3-month exposure period, suggesting that the length of exposure should be longer than 1 month, but exposure up to 3 months could fully damage the photosystem II (PSII) and destroy the moss bags. The vitality index (VI) also clearly indicated that the moss bags were more affected in the urban area, at the R sites, and at the 3-month exposure. Among all six parameters, the most susceptible parameter was the chlorophyll fluorescence parameter (Fv/Fm), followed by the total carotenoids, chlorophyll a, total chlorophylls, chlorophyll b, and chlorophyll a degradation (OD435/OD415). The moss bag technique used in this study did not negatively affect the studied physiological parameters, but a great elevation difference could affect the moss bag physiology. This study illustrated that the photosynthesis-related parameters of *L. aduncum* moss bags can be used as effective bioindicators of environmental stress caused by road traffic. This study is among the priority studies of moss bioindication of air pollution in Thailand and can pave the way for future studies.

ARTICLE HISTORY

Received: 1 Jul. 2023
Accepted: 16 Dec. 2023
Published: 23 Feb. 2024

KEYWORDS

Air monitoring;
Air pollution;
Physiological effect;
Vitality index;
Vehicle traffic

Introduction

Physiological response in sensitive organisms is often used as an early warning signal of environmental stress, especially air pollution [1–4]. Photosynthesis-related parameters, including chlorophyll fluorescence and photosynthetic pigment status, are frequently used [5–6]. Chlorophyll fluorescence is an easy and non-destructive technique used for evaluating the efficiency of photosystem II (PSII) in photochemical reactions [7]. Photosynthetic pigments, including chlorophylls and carotenoids, are used to estimate the size of light harvesting complexes (LHCs) and reaction centers (RCs) for capturing sunlight in the photosynthetic process. Chlorophyll degradation is estimated using the OD435/ OD415 ratio, which was originally proposed by Ronen and Galun [8] for estimating chlorophyll integrity in a lichen. This ratio evaluates the ship of chlorophyll a into phaeophytin a, in which a lower ratio indicates

https://doi.org/10.35762/AER.2024002

higher chlorophyll a degradation. These physiological parameters have been widely observed in mosses [9–10], lichens [11–12], and higher plants [13–15] to assess the effects of air pollution in areas of interest. They are simple, cost effective and reliable.

Terrestrial mosses are among the most effective bioindicators/biomonitors of air pollution. They are sensitive to air pollution [1, 16] and are great bio-accumulators of atmospheric deposition [17–21]. They have no root system and rely largely on the atmosphere for water and minerals (including pollutants). The lack of the cuticle layer allows atmospheric deposition to easily accumulate in their tissues. In addition, a large surface-to-mass ratio (SM ratio) increases their capacity for accumulating pollutants [16]. Air pollutants such as nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and some heavy metals are highly toxic to mosses. Early effects can be observed from changes in biochemical reactions, physiological processes, ultrastructure, and morphological abnormalities [1, 10, 16]. These effects are the consequence of the synergistic and/or additive effects of overall air pollution of the area, not from a single pollutant or a group of air pollutants. Therefore, the physiological response in moss samples can provide a good preliminary overview of the state of air quality in an investigated area. There are two types of biomonitoring using mosses. Firstly, the passive biomonitoring technique, which involves the use of in situ or native moss samples [5, 20, 22]. This method is appropriate when the targeted mosses are widely distributed across the study area. Secondly, the active biomonitoring technique involves the use of moss transplants or moss bags. It is widely used in areas where the targeted mosses are rarely found and insufficient in the study area, such as highly urban and industrial areas [17–18, 23]. The active technique has some advantages over the passive one: i) it can be used in areas where the native mosses are scarce or insufficient, ii) the exposure time is known, and iii) it allows air monitoring at high resolution such that the mosses can be placed in areas as much as needed. Moss biomonitoring of air pollution has been recognized and used for more than 40 years, but its intensity is high in Europe [24]. The most commonly used species are *Sphagnum* spp., followed by *Hypnum cupressiforme*, *Pseudoscleropodium purum*, *Pleurozium schreberi*, *Hylocomium splendens*, and others [24]. These species were present and abundant in the study areas.

Overall air quality monitoring is a complex task and cannot be done by using electronic air monitoring equipment alone. These instruments can detect some pollutants, especially common air pollutants, i.e., carbon monoxide (CO), NO_2, SO_2, ozone (O_3), particulate matter (PM), and volatile organic compounds (VOCs). However, there are more than 500 pollutants in the environment, and they can be toxic to organisms, including humans. Most of these pollutants can be detected using biomonitoring tools. Surveying air quality using bioindicators in Thailand is not commonly known. Some previous studies used lichens [11, 25–28], leaves [29–30], and tree bark [31–32], and a few studies used mosses [33–34]. Lacking this knowledge can interrupt air quality monitoring in some areas and can take risks to the population, especially children, who are the most sensitive group. Therefore, the objective of this study was to test the efficiency of photosynthesis-related parameters in *Leucobryum aduncum* Dozy & Molk moss bags as bioindicators of environmental stress related to road traffic. The results of this study will be of benefit to air quality monitoring using bioindicators in Thailand and other regions.

Materials and methods

1) Study area

This study was carried out in three areas that had different air pollution loads, including forested (Fo), rural (Ru), and urban (Ur) areas (Table 1). The forested area was in Khao Yai National Park (KYNP), the rural area was in the agricultural area in Ongkharak District, Chachoengsao Province and Bang Nam Priao District, Nakhon Nayok Province, and the urban area was in Bangkok. Air pollution among these areas was different, and the highest pollution was in the urban area, followed by the rural and forested areas. This was estimated based on the number and size of nearby roads, traffic flow, and proximity to urban areas and industrial plants. Based on the literature, road traffic can release several air pollutants into the environment, such as CO, carbon dioxide (CO_2), oxides of nitrogen (NOx), SO_2, polycyclic aromatic hydrocarbons (PAHs), VOCs, PM, and potentially toxic elements (PTEs) [35–36]. Two different sites from each area were selected: one site was on the roadside, hereafter R site, and another site was away from the road and received fewer effects from the road, hereafter A site (Table 1). These two sites were used to observe and compare the effects of air pollution from road traffic. Another site in the dry evergreen forest at approximately 806 m above sea level (asl) where the moss samples were collected was used as a reference site (REF) for observing the effects of elevation and transplantation method (moss bag). The A site in the forested area (FoA) was used as a control because its elevation was closer to all studied sites than the REF. Mosses largely rely on climatic conditions, especially air humidity, and temperature, which probably change with elevational gradient.

Table 1 Description of the studied sites

Area	Point	Site code	Location	Latitude Longitude [a]	Elevation (m asl) [a]	Remark
Moss collecting source and reference site	Forest floor	REF	In a forest near the Khao Yai National Park palace, Nakhon Ratchasima Province	14°26'12.93"N 101°23'8.14"E	806	Located inside the dry evergreen forest and surrounded by trees with dense canopy, approximately 50 m from the palace, and 112 km from Ramkhamhaeng University, Bangkok.
Forested (Fo)	Away from the road (A) and control	FoA	In a forest near the entrance to Khao Yai National Park, Prachinburi Province	14°13'25.24"N 101°24'22.32"E	59	Located inside the dry evergreen forest and surrounded by trees with dense canopy, approximately 60 m from the nearby road and site FoR, 100 km from Ramkhamhaeng University, Bangkok.
	Roadside (R)	FoR	On the roadside near the entrance to Khao Yai National Park, Prachinburi Province	14°13'24.51"N 101°24'20.48"E	60	Located on the roadside of Route 3077, an open area, approximately 100 km from Ramkhamhaeng University, Bangkok. Air pollution can originate from motor vehicles traveling in the park.
Rural (Ru)	Away from the road (A)	RuA	At Ban Khlong 21 School, Chachoengsao Province	13°56'43.02"N 101° 1'4.57"E	1	Located in a rural area and surrounded by paddy fields, approximately 3.8 km from the main road (Route 3001), 7.2 km from site RuR, and 48 km from Ramkhamhaeng University, Bangkok.
	Roadside (R)	RuR	At Wat Sunthorn Pichitaram School, Nakhon Nayok Province	13°58'33.66"N 101°4'34.90"E	6	Located on the roadside of the main road (Route 3001), approximately 55 km from Ramkhamhaeng University, Bangkok. Air pollution can originate from motor vehicles traveling on Route 3001.
Urban (Ur)	Away from the road (A)	UrA	At Faculty of Science, Ramkhamhaeng University (Hua Mark), Bangkok	13°45'16.10"N 100°37'7.73"E	3	Located inside Ramkhamhaeng University (Hua Mark), Bangkok, approximately 200-500 m from the road network outside the university and 500 m from site UrR.
	Roadside (R)	UrR	On the roadside in front of Ramkhamhaeng University (Hua Mark), Bangkok	13°45'21.33"N 100°36'51.39"E	3	Located on the roadside of Ramkhamhaeng Road, near the intersection. Air pollution can originate from motor vehicles.

Remark: [a] Data were received from Google Map Pro.

Climatic conditions, including rainfall, air temperature, and air humidity, from December to March in the last 10 years in the forested and rural areas were observed at the Prachinburi meteorological station, approximately 19.5 km (forested) and 32–39 km (rural) away. Those in the urban area were observed at the Bangkok meteorological station, approximately 7 km away, and rain amounts at the REF were observed at the KYNP rain station, approximately 1.4 km away. As shown in Figure 1, the air temperature was lowest at KYNP, while that at Prachinburi and Bangkok was similar. The air temperature for KYNP was 19.9 °C, while the average air temperature during the last 10 years for Prachinburi and Bangkok was 28.0±0.9 °C and 28.2±0.8 °C. The air humidity was highest in KYNP, while that at Prachinburi was slightly lower than that at Bangkok. The air humidity for KYNP was 75%, while the average air humidity during the last 10 years for Prachinburi and Bangkok was 65±2.8 % and 69±1.9 %. The cumulative rainfall fluctuated, and the average values between December and March from 2013 to 2022 were 62±52 mm for KYNP, 42±59 mm for Prachinburi, and 83±66 mm for Bangkok.

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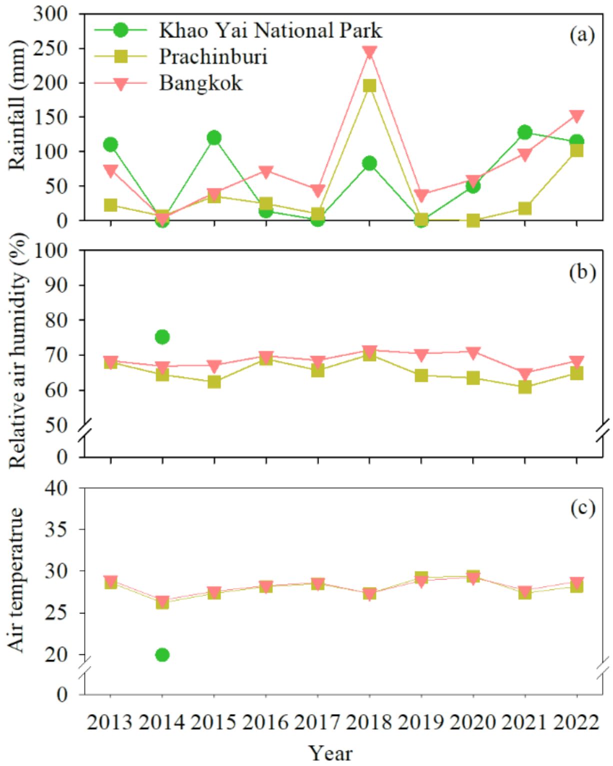

Figure 1 Cumulative rainfall (a), average air humidity (b), and average air temperature (c) between December and March from 2013 to 2022 at the Khao Yai National Park rain station (Code 431031, 14°26'3.89"N 101°22'21.24"E), Prachinburi meteorological station (Code 430201, 14°03'03.3"N 101°22'09.7"E), and Bangkok meteorological station (Code 455201, 13°43'28.5"N 100°33'48.0"E). The data for each year were calculated from 15 December (of the previous year) to 14 March (of consecutive year). Rain amounts at Khao Yai National Park were not recorded during 15–31 December 2012, 1–14 March 2017, 15–31 December 2019, and 1–14 March 2022 (The data was provided by the Thai Meteorological Department). Air humidity and temperature at Khao Yai National Park were recorded between 2013 and 2014 near the forest canopy, ca. 2 km away from the reference site by Mr. Mongkol Phaengphech (unpublished data) from the Lichen Research Unit.

2) Moss sample, exposure, and climate

Samples of the moss *L. aduncum* growing on the forest floor at the REF (unpolluted site) in KYNP (Figure 2a) were collected in December 2021. This moss was found in Southeast Asia, India, Papua New Guinea, Australia, New Zealand, and other Pacific Islands (https://www.gbif.org/species/2675704, accessed on 25 May 2023). It was previously used as a biomonitor of trace elements in Vietnam [37] and a radionuclide (^{210}Po) in Malaysia [38]. The moss samples were prepared in a relatively unpolluted area in KYNP. They were cleaned by washing and shaking in deionized water and then left to dry under natural air conditions. Approximately 500 mg of each moss sample was contained and spread in an 8 × 10 cm flat nylon bag with a 2 × 2 mm mesh size (weight/surface ratio of ca. 6.25 mg cm^{-2}), hereafter referred to as the "moss bag". The moss bag technique was designed based on the findings and suggestions by Ares et al. [24], Fern6ndez et al. [39], and Capozzi et al. [40]. Six bags were transplanted at each site by suspending on tree branches or PVP pipes approximately 3–5 m above the ground (Figure 2b), and a total of 42 bags were exposed at all sites. The moss bags were exposed for two different lengths of time in the dry season, 1 month (14 December 2021 to 12 January 2022) and 3 months (14 December 2021 to 13 March 2022). Three bags from each site and native (in situ) moss samples at the REF were collected at the end of each exposure period. The collection was performed within a day, each moss sample was contained in a polyethylene plastic zipped lock bag to prevent air contamination and kept under cool conditions during transfer to the laboratory. The samples were placed in a closed room under room temperature and humidity for about 15 h before physiological measurements.

Figure 2 The sample of moss *Leucobryum aduncum* growing on the forest floor at the reference site in Khao Yai National Park (a) and moss bags exposed at a study site (b).

3) Physiological measurement

All physiological measurements were performed within 24 h after collection in each period. The moss bags from each site and period were sorted in the laboratory. Only the apical parts of the moss shoot (ca. 1 cm from the tips) were chosen.

https://doi.org/10.35762/AER.2024002

The photosynthetic performance was estimated via the maximum quantum efficiency of PSII photochemistry or Fv/Fm = (Fm-Fo)/Fm [7]. The moss parts from each sample were immersed in deionized water for approximately 3-5 min to absorb water and then arranged on a plastic petri dish with a diameter of ca. 3 cm. Each sample was placed under dark conditions for 1 h and sprayed with deionized water every 15 min. The Fv/Fm was measured on well-wet samples using a MINI-PAM pulse amplitude modulated fluorometer (Heinz Walz GmbH, Effeltrich, Germany) that applied a saturating light pulse of ca. 4000–5000 μmol m^{-2} s^{-1} for 0.8 s under dark conditions and a controlled temperature room of ca. 25±2 °C. Six measurements were performed on each sample, and an arithmetic mean was calculated. Three samples (n=3) were measured for each site and period.

Approximately 50 to 70 mg of desiccator-dry weight of each moss sample was used for photosynthetic pigment extraction. The pigment extraction in the moss samples was adapted from the procedure by Barnes et al. [41]. The moss sample was immersed in 5 mL dimethyl sulfoxide (DMSO), which contained 2.5 mg mL^{-1} polyvinylpyrrolidone that helped to prevent chlorophyll degradation during the extraction process, and then incubated in a hot air oven at 65 °C for 45 min in darkness. After that, the extract was allowed to cool to ambient temperature, and another 5 mL of DMSO was added and left in the dark for ca. 15 h. The optical density of the extract solution was measured at wavelengths of 415, 435, 480, 649 and 665 nm with a spectrophotometer (GENESYS 10S UV–Vis spectrophotometer, Thermo Fisher Scientific Inc., USA) to determine chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl), total carotenoid (TCar), and chlorophyll degradation (OD435/OD415) content. The equations by Wellburn [42] were used to calculate the pigment contents. Three measurements were performed on each sample, and an arithmetic mean was calculated. Three samples (n=3) were measured for each site and period.

4) Statistical analysis

The dataset of each physiological parameter was checked for normality and homogeneity of variance before selecting statistical tests. The normality was tested using Shapiro–Wilk's test ($p<0.05$), and homogeneity of variance was tested using Levene's tests ($p<0.05$). A dataset with normal distribution and equal variance were examined using t-test or one-way analysis of variance (one-way ANOVA) with Tukey's test for post hoc comparison. While that with non-normal distribution and unequal variance were examined using the Kruskal–Wallis test with Dunn's test for post hoc comparison. All statistical tests were performed using the Real Statistics Resource Pack software (Rel 8.6.2, Charles Zaiontz, www.real-statistics.com).

Overall moss vitality at each site and period was evaluated using a vitality index (VI); $VI = (SF_1 \times SF_2 \times SF_3 \times \ldots \times SF_n)^{1/n}$, where n is the number of studied physiological parameters. SF is a stress factor; SF = Pm/Pc, where Pm is the average value of a physiological parameter at a study site, and Pc is the average value of the same physiological parameter from all exposure periods at the control site [11].

Results and discussion

The results of this study confirm that the studied physiological parameters, including Fv/Fm, Chl a, Chl b, TChl, TCar, and OD435/OD415, in *L. aduncum* moss bags can be used as effective bioindicators of the effects of air pollution caused by road transport in the study areas. Because the amounts of rainfall, relative air humidity, and air temperature during the study period were not much different among all three study areas (Figure 1), the physiological response of the moss bags was probably influenced mainly by surrounding air pollution. The Fv/Fm showed clear trends of the effects of air pollution on both exposure periods, where the highest average values from the A and R sites were observed in the forested area, followed by the rural and urban areas. The average Fv/Fm values at the urban sites were significantly lower than those at the forested sites in both exposure periods (Figure 3a-b). Meanwhile, the other parameters, including Chl a, Chl b, TChl, TCar, and OD435/OD415, showed such trends at the 3-month exposure (Figure 3c-l). This may suggest that the exposure time to study the effects of air pollution on the physiological parameters of this moss species should be longer than 1 month. However, an exposure time of more than 3 months could fully damage the PSII and destroy the moss bags in the urban area and at the R site in the rural area, as suggested by the Fv/Fm parameter. This parameter is generally used to check the status of photosynthetic organisms. The samples that received optimal water content and showed the Fv/Fm value of zero indicate dead samples. Most studies on biomonitoring using moss bags took 30 to 60 days [24]. Ares et al. [24] suggested 45 days, and Capozzi et al. [40] advised exposing moss bags not shorter than 6 weeks.

The effects of air pollution on the physiological parameters of the moss bags were further observed from the different values between sites A and R (Figure 3). All parameters at most R sites were lower than those at A sites in each area, confirming the higher effects at R sites (the roadside sites). At the 3-month exposure, all parameters except Chl b at the rural R sites showed significantly lower values than those at the rural A sites, which probably indicates the different air quality between the sites. All parameters at the A and R sites in the urban area showed close values and had no statistically significant differences. This might suggest that the moss bags at the urban sites were equally affected after exposure for 3 months. However, no significant differences at the A and R sites in the forested area were probably caused by low air pollution at the R site.

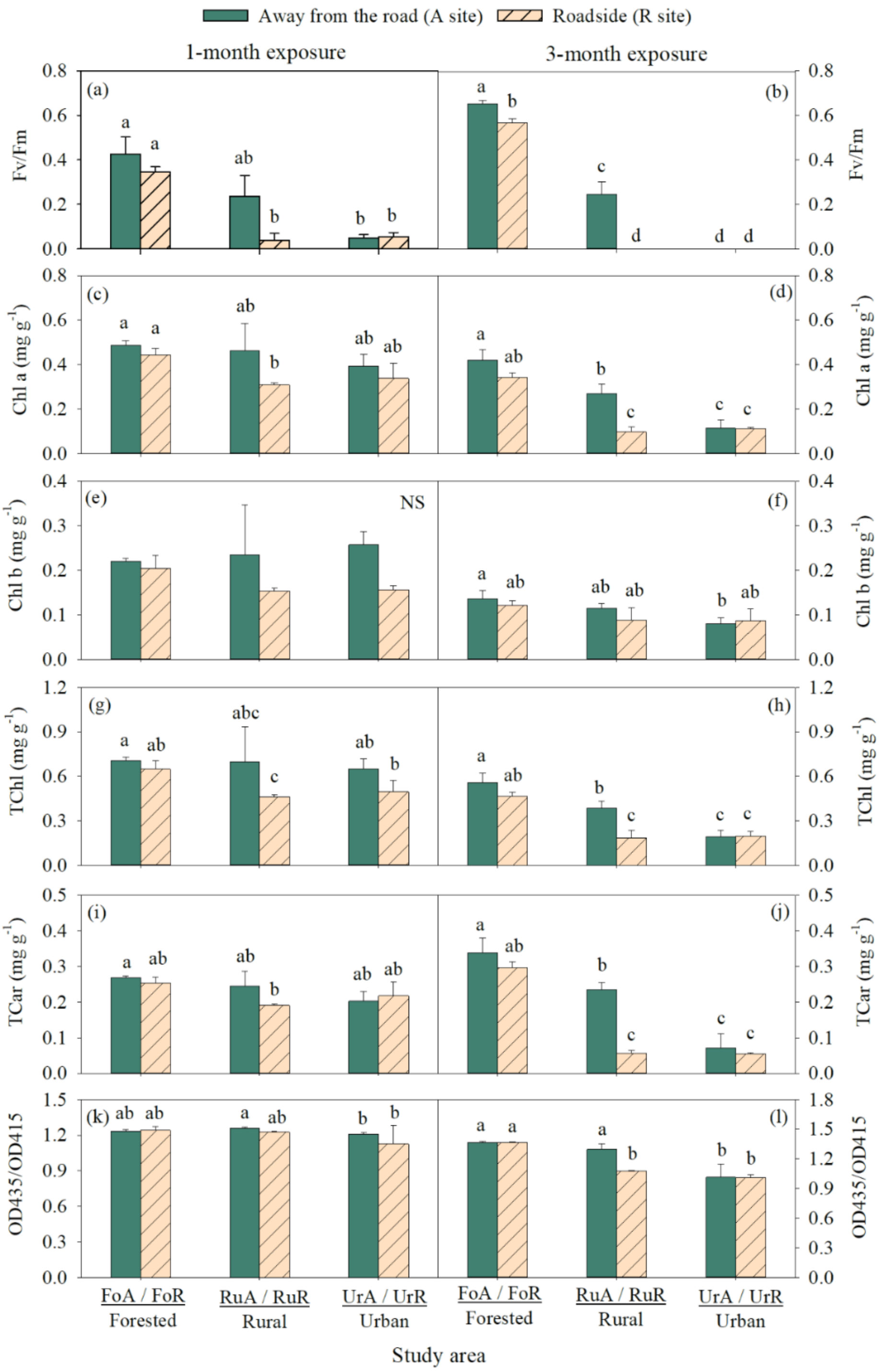

Figure 3 Means and standard deviations (n=3) of the physiological parameters including the maximum quantum efficiency of PSII photochemistry (Fv/Fm, a, b), chlorophyll a (Chl a, c, d), chlorophyll b (Chl b, e, f), total chlorophylls (TChl, g, h), total carotenoids (TCar, i, j) and chlorophyll degradation (OD435/OD415, k, l) of the *Leucobryum aduncum* moss bags in the forested, rural, and urban areas during 1- and 3-month exposures. Different letters shown on each panel indicate statistically significant differences by one-way analysis of variance with Tukey's test for post hoc comparison (1-month: Chl b, TCar, and 3-month: Fv/Fm, Chl a, Chl b, TChl, TCar, OD435/OD415) and by Kruskal–Wallis test with Dunn's test for post hoc comparison (1-month: Fv/Fm, Chl a, TChl, OD435/OD415) at $p<0.05$, NS = no significance.

The overall moss bag vitality at each site and exposure period based on the six studied physiological parameters were evaluated using a VI (Figure 4). This index was previously used to evaluate the vitality of lichens exposed in industrial areas, for which a higher VI value indicates the better health of the samples. [11, 43]. The VI values of the *L. aduncum* moss bags also showed lower values at the suspected higher air pollution sites. The moss bags at all sites exposed for 3 months had lower VIs than those exposed for 1 month; however, the VIs at the control site differed slightly between the exposure periods. At the 3-month exposure, the VIs in the urban sites showed lower values than those in the rural and forested sites, except for the rural R site (RuR), which had an equal value. The VIs at all R sites were also lower than their A sites in all areas and periods, except the urban area at the 3-month exposure, which showed similar values.

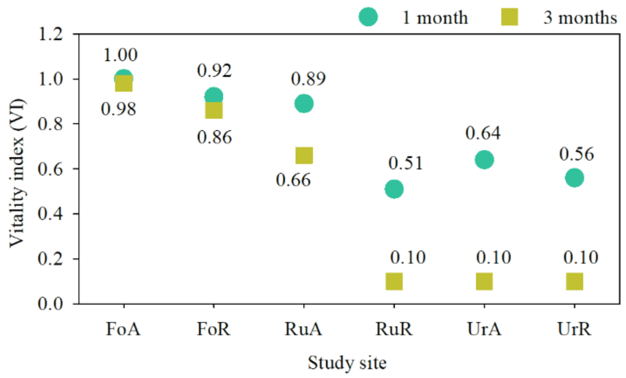

Figure 4 The vitality index (VI) of the *Leucobryum aduncum* moss bags at all sites during 1- and 3-month exposures, calculated using all six studied physiological parameters.

The results of this study corresponded to those of previous studies. For example, Swislowski et al. [44] observed that the concentrations of Chl a in the moss *Pleurozium schreberi* transplanted in the center of Opole city, Poland, decreased with longer exposure time, while Chl b fluctuated and did not show a clear trend. Sujetoviene and Galinyte [3] found that Chl a, Chl b, and Fv/Fm in *Ptilium crista-castrensis* moss bags were lower in urban, suburban, and residential areas than in the control site, especially after 2 months of exposure. Tretiach et al. [1] reported that Chl a, Chl b, TChl, TCar, Fv/Fm, and net photosynthesis (Pn) in *Hypnum cupressiforme* moss bags decreased after 6 weeks of exposure in the urban areas of Trieste and Naples in Italy. Varela et al. [10] revealed that the chlorophyll content index and growth rates in the moss *Pseudoscleropodium purum* transplanted at different distances from a steelworks factory in Spain were lowest at the high concentrations of metals and metalloids in the moss tissues. Tremper et al. [9] found that Cu caused a significant decrease in the total chlorophyll content in the moss *Rhytidiadelphus squarrosus* in a laboratory experiment. In addition, the effects of heavy metals on moss physiology, anatomy, and morphology were documented in the review by Stankovic et al. [16].

To find the most susceptible parameter among the studied physiological parameters in the moss bags, the data from the R sites in each area, the higher polluted sites, were used to compare with the control site (FoA). The most susceptible parameter was Fv/Fm, followed by TCar, Chl a, TChl, Chl b, and OD435/OD415. This was suggested by the average remaining percent integrity at the R sites in all areas compared to the control site (Figure 5), which were 29% for Fv/Fm, 40% for TCar, 44% for Chl a, 51% for TChl, 72% for Chl b, and 84% for OD435/OD415. This was in accordance with the findings in the transplanted lichen *Parmotrema tinctorum* [11] and *Ramalina lacera* [45]. It is beneficial for the selection of physiological parameters for observing the effects of environmental stress.

This study also investigated the effects of the moss bag technique on physiological parameters. The studied physiological parameters of the in situ moss samples varied after exposure, however these samples were in the forest with no pollution thus different values of the physiological parameter were assumed from the climate. As shown in Figure 6, all parameters in the moss bags showed significantly higher values than those in the in situ moss samples, except for Fv/Fm after 1 month of exposure. This confirms that the moss bag technique did not adversely affect the physiological parameters. In addition, this work also revealed that a great elevation difference could affect all physiological parameters after 3 months of exposure (Figure 7). The Chl a, Chl b, TChl, TCar, and OD435/OD415 in the moss bags at the lower elevation site (FoA, 59 m asl) had significantly lower values than those at the higher elevation site (REF, 806 m asl). This was probably because of the different climatic conditions, especially air humidity and temperature. The average relative air humidity and temperature for the REF were 75% and 19.9 C and 65% and 28.0 C for the FoA (Figure 1). This suggests that the selection of a control site should be located at similar or close elevations to avoid confounding factors by such climatic conditions.

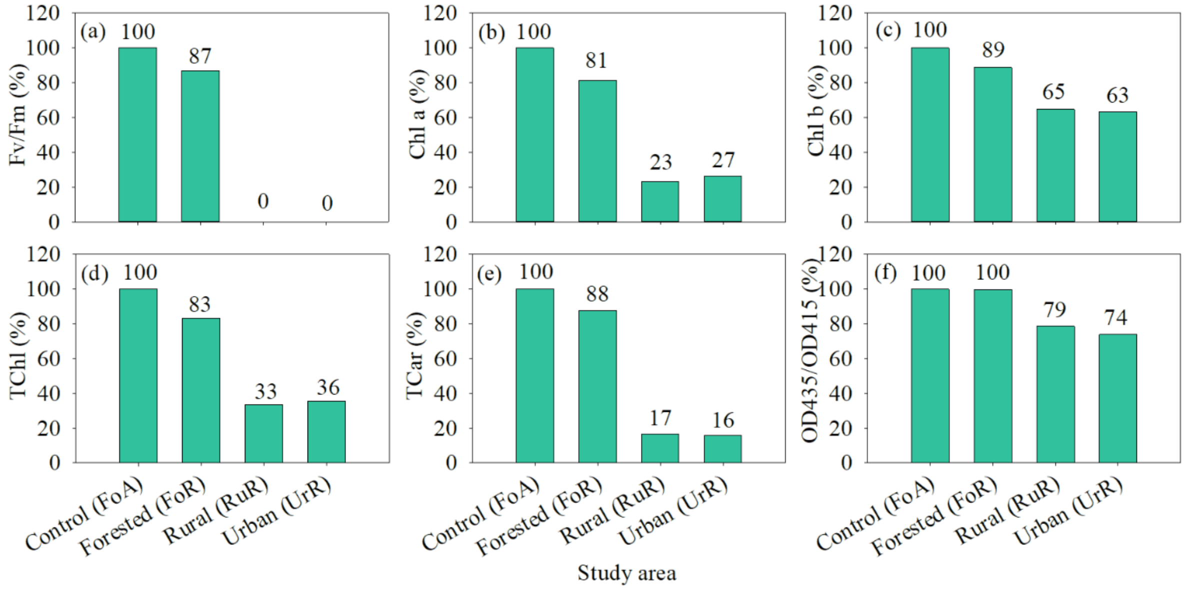

Figure 5 The remaining percent integrity compared to the control site of each physiological parameter, including the maximum quantum efficiency of PSII photochemistry (Fv/Fm, a), chlorophyll a (Chl a, b), chlorophyll b (Chl b, c), total chlorophylls (TChl, d), total carotenoids (TCar, e) and chlorophyll degradation (OD435/OD415, f), of the *Leucobryum aduncum* moss bags after 3 months of exposure at the R sites in each area.

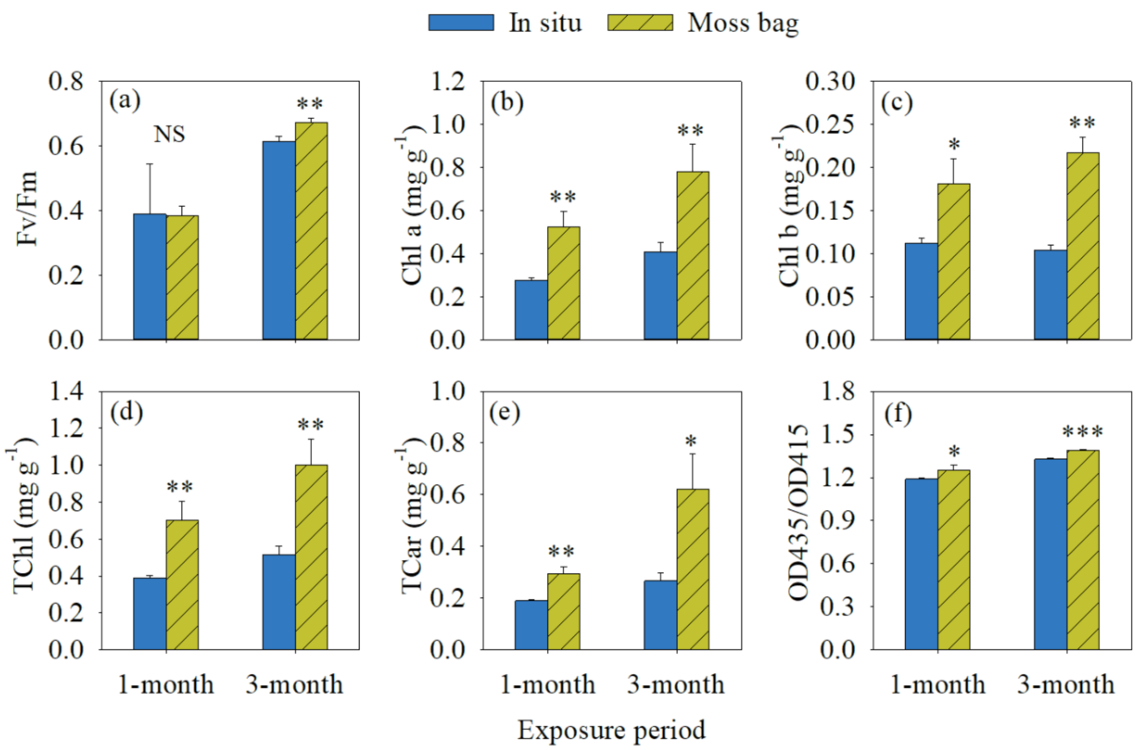

Figure 6 Means and standard deviations (n=3) of the physiological parameters, including the maximum quantum efficiency of PSII photochemistry (Fv/Fm, a), chlorophyll a (Chl a, b), c hlorophyll b (Chl b, c), total chlorophylls (TChl, d), total carotenoids (TCar, e) and chlorophyll degradation (OD435/OD415, f), of the in situ and moss bags of *Leucobryum aduncum* at the reference unpolluted site (REF) in Khao Yai National Park during 1- and 3-month exposures. Asterisks on each pair indicate statistically significant differences by t-test, *p<0.05, **p<0.01, ***p<0.001, NS = no significance.

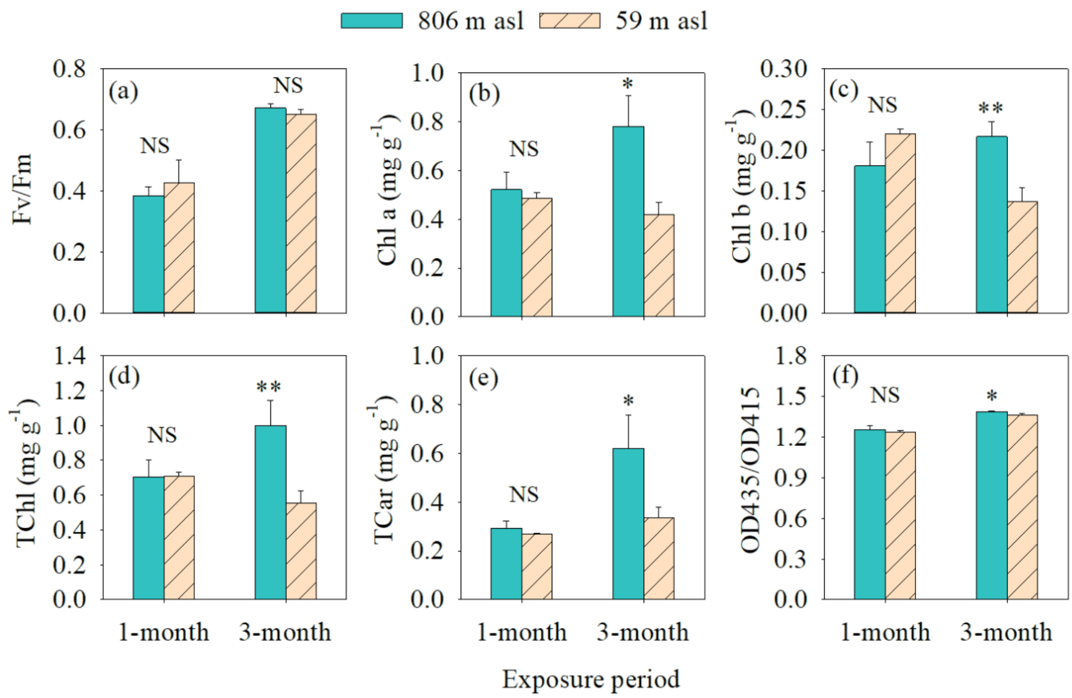

Figure 7 Means and standard deviations (n=3) of the physiological parameters including the maximum quantum efficiency of PSII photochemistry (Fv/Fm, a), chlorophyll a (Chl a, b), chlorophyll b (Chl b, c), total chlorophylls (TChl, d), total carotenoids (TCar, e) and chlorophyll degradation (OD435/OD415, f) of the *Leucobryum aduncum* moss bags at elevations of 806 m asl (REF) and 59 m asl (FoA) in Khao Yai National Park during 1- and 3-month exposures. Asterisks on each pair indicate statistically significant differences by t-test, *$p<0.05$, **$p<0.01$, NS = no significance.

The findings of this study can be beneficial for bioindication studies of air pollution using physiological responses in moss samples. The physiological parameters used in this study are easy to measure, inexpensive, and provide low variation results; thus, they are very useful for all investigators who are interested in this aspect. In addition, the results obtained by this study can be applied to bioindication studies using other photosynthetic organisms, such as higher plants and lichens. Mosses are among the best bioaccumulators of PTEs [46–48], PAHs [49–51], radionuclides [38], and airborne microplastics [52–53]; therefore, they can be used as complementary tools to traditional air quality monitoring instruments for assessing air quality.

Conclusions

The photosynthesis-related parameters in the *L. aduncum* moss bags were demonstrated to be effective bioindicators of environmental stress caused by road traffic in the study areas. The average value of each parameter was highest in the forested area and lowest in the urban area. This sequence was particularly observed in the 3-month exposure period, suggesting that the length of exposure should be longer than 1 month, but exposure up to 3 months could fully damage the PSII and destroy the moss bags. All parameters at most R sites were lower or significantly lower than those at their A sites, confirming the higher effects at the R sites, the roadside sites. Accordingly, the VI values also clearly indicated that the moss bags were more affected in the urban area, at the R sites, and during the 3-month exposure period. Among all six parameters, the most susceptible parameter was Fv/Fm, followed by TCar, Chl a, TChl, Chl b, and OD435/OD415. The moss bag technique used in this study did not negatively affect the physiological parameters of the moss samples; thus, it can be utilized in future studies. A great elevation difference could affect the moss bag physiology, which was probably due to different climatic conditions, especially air humidity and temperature. The physiological parameters used in this study are simple, inexpensive, and provide reliable results; thus, they are very useful for bioindication studies of air pollution, especially in Thailand. This study is among the priority studies of moss bioindication of air pollution in Thailand and can pave the way for future studies.

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