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RESEARCH ARTICLE

Lichen biomonitoring of seasonal outdoor air quality at schools in an industrial city in Thailand

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Abstract
Poor air quality in school environments causes adverse health effects in children and decreases their academic performance. The main objective of this study was to use lichens as a biomonitoring tool for assessing outdoor air quality at schools in the industrial area of Laem Chabang municipality in Thailand. Thalli of the lichen *Parmotrema tinctorum* were transplanted from an unpolluted area to nine schools in the industrial area and to a control site. The lichens were exposed for four periods in the dry, hot, early rainy, and late rainy seasons, for 90 days each. The concentrations of 14 elements, including As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Ti, V, and Zn, were determined using inductively coupled plasma–mass spectrometry (ICP–MS), and 8 physiological parameters were measured. The concentrations of all 14 investigated elements were clearly higher at the schools than at the control site. The contamination factors (CFs) suggested that 9 out of the 14 elements, including As, Cd, Co, Cr, Cu, Mo, Pb, Sb, and Ti, heavily contaminated the school environments, especially Pb, the concentration of which was 3 to 11 times higher than at the control site. The most polluted time was the hot season as evidenced by the investigated elements, and the least polluted time was the late rainy season. The pollution load indices (PLIs) demonstrated that schools in the inner and middle zones clearly had higher pollution loads than the schools in the outer zone during the rainy seasons, while the hot and dry seasons showed similar pollution levels in all zones. The vitality indices (VIs) showed that the lower lichen vitalities at most schools were observed during the dry season and at the schools in the inner and middle zones. Accordingly, the air performance indices (APIs) revealed that poorer air quality at most schools was found during the dry season and at the schools in the inner and middle zones. This study clearly showed that the transplanted lichen *P. tinctorum* was an effective bioindicator of air quality in school environments. The results illustrated that all studied schools were contaminated by air pollutants; therefore, improving air quality at the schools is crucial and should be an urgent issue for maintaining good health and may benefit children's academic achievements and careers in the long run.

Keywords Air performance index · Air pollution · Bioindicator · Laem Chabang · Lichen transplant · *Parmotrema tinctorum* · Physiological parameter · Trace element

Introduction

Industries are sources of several kinds of air pollutants, including oxides of nitrogen (NO_x), oxides of sulfur (SO_x), carbon monoxide (CO), carbon dioxide (CO_2), ozone (O_3), particulate matter (PM), heavy metals, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs) (Nadal et al. 2009; Parviainen et al. 2019; Pimpisut et al. 2005). These pollutants are emitted from fossil fuel combustion, manufacturing processes, and distribution of the products, as well as nearby road traffic. Children and elders are the most susceptible to air pollution (Makri and Stilianakis 2008). Exposure to poor air quality has been linked to respiratory

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disease, cardiovascular disease, skin diseases, lung cancer, allergies, and diabetes (Bergstra et al. 2018; Kim et al. 2016; Oliveira et al. 2019; Osborne et al. 2021a). Outdoor and indoor air quality at schools are particularly environmental concerns, especially schools located in highly polluted areas, i.e., industrial and urban areas (Amato et al. 2014; Cai et al. 2021; Guo et al. 2010; Villanueva et al. 2018). Children are one of the most vulnerable groups to air pollution because i) their respiratory, immune, reproductive, central nervous, and digestive systems are not fully developed; ii) their inhalation rates per body weight are higher than those of adults; and iii) they spend much of their time in school environments (up to 10 h daily) (Oliveira et al. 2019). Apart from serious health effects, air pollution can also decrease children's academic performance (Lu et al. 2021; Requia et al. 2021a; Rojas-Vallejos et al. 2021); therefore, monitoring air quality in school environments is a crucial task.

Air quality is generally measured using electronic instruments. This method has a relatively high cost, and it is a constraint for air monitoring in low- and middle-income countries. In Thailand, most of the provinces have only one air quality monitoring station, and the air quality of the whole province is generally based on the data from the station. Air pollution varies greatly with space and time, and assessing the air quality of a whole area using data from one point can increase health risk. School environments should be given more attention and probably do not rely on the air quality monitoring station alone, especially where they are far from the station. Ideally, each school should have its own air pollution surveillance for further safety. Biomonitoring is a cost-effective method and can be used as a complementary tool for air quality assessment (Nimis et al. 2002). Lichens are obligate symbiotic organisms between fungi and algae and/or cyanobacteria (Nash 2008a). They are well known as effective bioindicators of air quality and great bioaccumulators of atmospheric pollutants (Abas 2021; Bargagli and Mikhailova 2002; Brunialti and Frati 2014). They have no root system and receive water and minerals from the air only. The absence of stomata and cuticle allows atmospheric deposition to accumulate on the whole lichen surface. One lichen thallus can accumulate several kinds of pollutants, especially trace elements that are generally not monitored at traditional air quality monitoring stations (Cecconi et al. 2021; Incerti et al. 2017; Zhao et al. 2019). Lichens are among the most sensitive organisms to air pollution; therefore, alterations in their physiology, anatomy, and morphology can be used as early warning signs of the effects of air pollution (Paoli et al. 2015; Sujetovienė et al. 2020). In a study area with no lichens or insufficient, this can be overcome by the transplantation technique (Brunialti and Frati 2014). This method has several advantages: i) it allows the assessment of spatial and temporal air pollution, ii) the exposure time is known and can be used to calculate enrichment or accumulation factors, and iii) it allows air monitoring at high resolution such that the lichens can be placed in areas of interest as much as needed. The lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale has great potential for use as a bioindicator/biomonitor/bioaccumulator of air pollutants. It was previously utilized for air quality assessment in Brazil (Palharini et al. 2021; Port et al. 2018), Japan (Dohi et al. 2015; Ohmura et al. 2015), India (Daimari et al. 2021), Malaysia (Zulaini et al. 2019), and Thailand (Boonpeng et al. 2017, 2020).

Laem Chabang (LCB) municipality is in Chonburi Province on the eastern coast of Thailand. This is an important port city for Thailand. Air pollution in this area can originate from industrial plants, petrochemical plants, industrial ports, transportation, and road traffic. There is one air quality monitoring station in the area operated by the Pollution Control Department of Thailand (http://air4thai.pcd.go.th/webV2/). Although there are several emission sources, we did not find any research about air pollution in the LCB area. There are 17 schools located in LCB, most of which are kindergarten and primary schools with students aged between 4 and 12 years (https://www.lcb.go.th/frontpage). Children are one of the populations that are most sensitive to air pollution, and they spend much of their time in school environments. The class time of most schools in Thailand is between 8.30 and 15.30. Therefore, air monitoring in schools is of paramount importance to protect children's health and maintain their academic performance. Research on school air quality has mostly been conducted in high-income countries (Osborne et al. 2021a), especially in Europe (Osborne et al. 2021b; Villanueva et al. 2018) and North America (Berman et al. 2018; Lu et al. 2021). Few studies on air quality in schools have been performed in low- and middle-income countries (Cai et al. 2021; Godoi et al. 2013; Requia et al. 2021a). Therefore, there is a need for many studies in low- and middle-income countries due to challenges with air pollution. In addition, most of the studies focused on the effects of PM and NO_2 (Lu et al. 2021; Osborne et al. 2021a, 2021b), some studies focused on VOCs, CO, CO_2, and O_3 (Cai et al. 2021; Godoi et al. 2013; Villanueva et al. 2018), and a few studies focused on heavy metals (Al-Hemoud et al. 2017). In Thailand, studies focused on school air quality and student health caused by air pollution are scarce (Moondee et al. 2004; Phantu and Bootdee 2022). Air pollution research at schools in LCB has not been detected before; therefore, the results of this study will benefit the management of school environments and urban planning. Although lichens are recognized as effective bioindicators of air quality, lichen biomonitoring in schools is sparse. Only 4 studies were found from Portugal (Canha et al. 2012, 2014), Italy (Protano et al. 2017), and Slovakia (Paoli et al. 2019a); therefore, increasing studies on this aspect will promote the use of lichens for assessing school air quality. The objectives of this study were i) to use lichens as biomonitors of airborne trace elements in school environments, ii) to study the physiological response of lichen transplants to assess the effects of air pollution on organisms, and iii) to preliminarily assess outdoor air quality at schools in the LCB industrial area.

Materials and methods

Study area and monitoring site

This study was carried out in school environments in the LCB municipality, which covers an area of approximately 109.65 km^2 and is located on the eastern coastline in Chon Buri Province, Thailand (Fig. 1). Under the Eastern Seaboard project, this area has been changed from a fishery community to an industrial area since 1982. Most parts of the area are plains with elevations ranging from 3 to 52 m above sea level (m asl). In January 2022, the registered population was 90,524 persons, and the nonregistered population was more than 60,000 persons. There are three seasons, including dry (mid-November to mid-February), hot (mid-February to mid-May), and rainy (mid-May to mid-November) seasons. For twenty-nine years (1992–2020), the average yearly cumulative rainfall was 1116 mm. Rain of more than 100 mm/month was observed between May and October. Heavy rain occurred from September to October (> 200 mm/month), and light rain occurred from December to February (< 20 mm/month). The average air relative humidity ranged from 62 to 79%, in which the most humid was in September and the least humid was in December. The monthly average temperature ranged from 28.0 to 30.1, with April being the hottest month and December being the coldest month. The prevailing winds blow from the southwest in the hot and rainy seasons and from the east in the dry season (source: Thai Meteorological Department). There were more than 200 companies in the Laem Chabang industrial estate (LCBIE), working related to automobiles, electronics, chemicals, paints, metallurgical plants, food, and beverages. Approximately 80 factories were in the Sahapat industrial park (SIP) that worked mostly on food and beverages. There are three oil refineries, three crude oil zones, three natural gas zones, and a Laem Chabang industrial port (LCBIP) (https://www.lcb.go.th/frontpage). Two major roads (Rout 3 and 7) passed through the area.

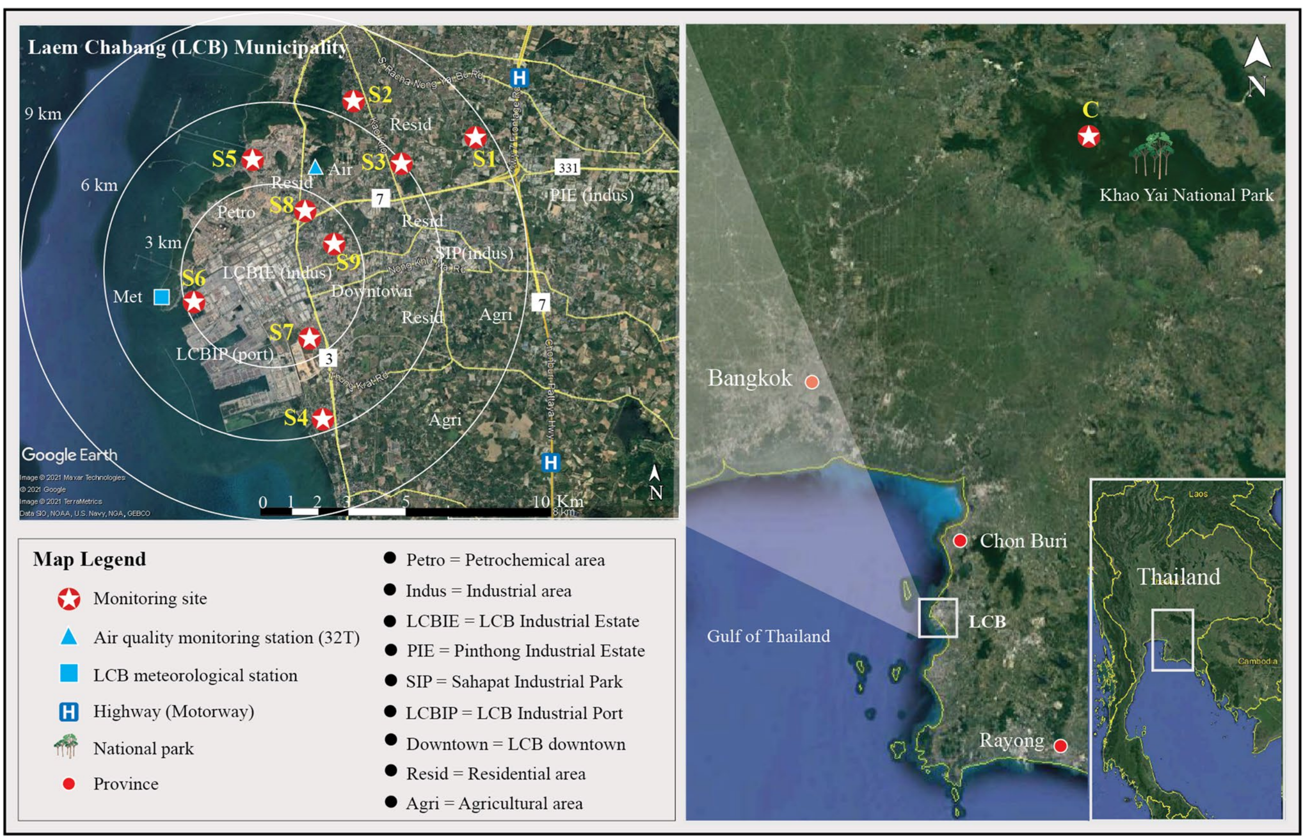

Fig. 1 Study area and monitoring sites (S1–S9) in Laem Chabang municipality in Chon Buri Province, Thailand, and the control site (C) in the unpolluted area at Khao Yai National Park

Air pollution in this area can originate from industrial processes and transportation by automobiles and ships. Data from the air quality monitoring station (32 T) by the Pollution Control Department of Thailand during the last 10 years (2011 to 2020) revealed that concentrations of CO, NO_2, and SO_2 never exceeded the national standards, while the PM10 (particulate matter with a diameter of 10 µm or less) was 3 of 2974 measuring times (0.10%), PM2.5 (particulate matter with a diameter of 2.5 µm or less) was 70 of 1822 measuring times (3.84%), and O_3 was ca. 0.8% exceeded their national standards (http://air4thai.pcd.go.th/webV2/download.php).

Nine primary schools were selected as the representative monitoring sites in the LCB area (S1–S9), and one control site (C) was in the unpolluted site at Khao Yai National Park (Fig. 1 and Table 1). The school monitoring sites were chosen based on the possibility of receiving effects of air pollution, i.e., adjacent to suspected emission sources (factory, port, and road), located downwind of the industrial plants, and student's ages below 15 years (vulnerable group). The study area was divided into three zones based on the distance

Table 1 Description of monitoring sites (S1–S9) in Laem Chabang municipality in Chon Buri Province, Thailand, and the control site (C) in the unpolluted area at Khao Yai National Park

Monitoring site	Location	Coordination[a]	Elevation (m asl)[a]	Student's ages (years)[b]	Number of students (persons)[b]	Remarks
Control (C)	Khao Yai National Park (KY)	14°25′12″N 101°22′44″E	752	-	-	The relatively unpolluted area in Khao Yai National Park, ca. 156 km northeast of the center of LCBIE
School 1 (S1)	Wat Nongkham School (WNK)	13°7′48″N 100°58′16″E	25	4–12	650	In the outer zone, ca. 8.3 km northeast of center of LCBIE, but ca. 2 km northwest of the Pinthong industrial estate (PIE) and the highway (road 7)
School 2 (S2)	Wat Phibulsanhatum School (WPB)	13°8′22″N 100°56′16″E	52	4–12	597	In the outer zone, ca. 6.7 km northeast of the center of LCBIE
School 3 (S3)	Wat Phrapratanporn School (WPP)	13°7′10″N 100°56′47″E	35	5–12	255	In the middle zone, ca. 5.5 km northeast of the center of LCBIE
School 4 (S4)	Ban Banglamung School (BBL)	13°2′43″N 100°55′30″E	3	5–12	138	In the middle zone, ca. 5.0 km south of the center of LCBIE, and close to the LCBIP
School 5 (S5)	Wat Mai Nernpayom School (WMN)	13°7′22″N 100°53′53″E	8	5–12	461	In the middle zone, ca. 4.0 km north of the LCBIE, but close to the petrochemical area
School 6 (S6)	Wat Laem Chabang School (WLC)	13°4′53″N 100°52′56″E	5	5–15	250	In the inner zone, ca. 2.8 km west of the center of LCBIE, but close to the LCBIP, LCBIE and the petrochemical area
School 7 (S7)	Wat Banna School (WBN)	13°4′12″N 100°55′17″E	7	4–12	964	In the inner zone, ca. 2.4 km southeast of the center of LCBIE, and close the LCBIP, and road 3
School 8 (S8)	Wat Manorom School (WMR)	13°6′25″N 100°54′59″E	23	5–15	1123	In the inner zone, ca. 2.35 km northeast of the center of LCBIE, and close to the petrochemical area, road 3 and 7
School 9 (S9)	Laem Chabang 2 Municipality School (LCM)	13°5′37″N 100°55′41″E	40	4–12	> 1500	In the inner zone, ca. 2.30 km northeast the center of LCBIE, and located in the downtown area

[a]Data was obtained from the Google Earth Pro

[b]Data were retrieved from the Education Management Information System (EMIS), http://data.bopp-obec.info/emis/index_area.php?Area_CODE=2003, updated 25 June 2021

from the center of the LCBIE. The outer zone, which was 6–9 km away, was composed of S1 and S2. The middle zone, which was 3–6 km away, comprised S3, S4, and S5. The inner zone, which was within 3 km, included S6, S7, S8, and S9.

Lichen sample and exposure time

The epiphytic foliose lichen *P. tinctorum* was selected as a biomonitoring tool for this study (Fig. S1 a). It is a cosmopolitan species (GBIF.org) found in mild to high humidity mountain regions across Thailand. This lichen was chosen because i) its population size was large enough for utilization; ii) it was easy to identify and prepare; iii) it had a high surface/mass (SM) ratio, which contributes to a higher capacity of element accumulation; iv) it had a relatively rapid growth rate among the lichens (15–30 mm/yr, (Fuangkeaw 2018; Wannalux 2014)); and v) its potential for bioindication/biomonitoring/bioaccumulation of air pollutants was proven by several studies in Brazil (Koch et al. 2016; Palharini et al. 2021; Port et al. 2018), Japan (Ohmura et al. 2009), India (Daimari et al. 2021), and Thailand (Boonpeng et al. 2017, 2020, 2018). Thalli of the lichen were collected on the bark of several phorophytes (host trees) in the unpolluted area at Khao Yai National Park at elevations ranging from 700 to 800 m asl by using stainless knives. All lichen thalli were checked for species correction and then cleaned by removing the other parts, such as other lichen species, bark, debris, dirt, mosses, and insects. Approximately 10 cm^2 of each thallus was fixed on an 8 × 8 cm polyethylene plastic netting (2 × 2 mm mesh size) by using a fishing line. All samples were then placed in a nursery area for physiological adaptation and homogeneity of approximately 45 to 60 days before being exposed at the monitoring sites.

At each exposure time, each lichen sample was picked from the nursery and washed by submergence and shaking in about 100 mL deionized water (DI), approximately 2–3 min to clean and increase homogeneity in terms of element composition (Boonpeng et al. 2021, Fig. S1 b). The washed lichen thalli were air dried overnight. Five thalli were contained in a 50 × 10 × 10 cm polyethylene cage (8 × 8 mm mesh size) and placed 30° to 45° for inclination (Fig. S1 c). Five cages were hung on suitable tree branches or PVC pipelines (if there were no suitable tree branches) (Fig. S1 d). The exposures were performed in four periods/seasons (90 days each) between 2 December 2018 and 29 November 2019. The first exposure was from 2 December 2018 to 1 March 2019 according to the cool dry season, the second exposure was from 3 March 2019 to 31 May 2019 according to the hot season, the third exposure was from 2 June 2019 to 30 August 2019 according to the early rainy season, and the fourth exposure was from 1 September 2019 to 29 November 2019 according to the late rainy season. During the study period, rainfall was lowest in the dry season (32 mm) and highest in the late rainy season (320 mm) (Fig. S2). The relative humidity was not very different, ranging from 67 to 73%, as was the temperature, which ranged from 29.6 to 31.2 °C (Fig. S2). Most wind blows from the southeast direction, and some eastern winds were found in the dry and late rainy seasons (Fig. S3). The concentrations of NO_2 and SO_2 were much lower than the national standards, while O_3 was only 2 times higher than the national standard in the dry season. Concentrations of PM10 and PM2.5 were highest and exceeded the national standards in the dry season (Fig. S4 and Table S1).

Element analysis

The lichen samples were dry cleaning to remove debris and dirt from the lichen surfaces. The analysis of element concentrations in the lichen samples followed Sangiamdee (2014) and Boonpeng et al. (2021). Briefly, the unwashed exposed lichen sample was immersed in liquid nitrogen and subsequently pulverized and homogenized with a ceramic mortar and pestle. It was then separated through a 500-µm sieve plate. Approximately two hundred milligrams of the lichen powder was mineralized with 4 mL of conc. HNO_3 in a microwave digestion system (AIM 600, Aim Lab, Australia) at 140 °C for 40 min. The concentrations of fourteen elements, including As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Ti, V, and Zn, were determined using inductively coupled plasma−mass spectrometry (ICP−MS, NexION 300Q, PerkinElmer, USA). The analytical quality was assessed with the certified reference material BCR-482 and spike samples. The recoveries ($n = 7$) ranged between 92.8 (V) and 101.8% (Fe). The analytical precision, expressed as percent relative standard deviation (%RSD), was less than 6% ($n = 7$) for all analyzed metals.

Physiological measurement

Net photosynthesis

The physiological measurement followed the procedure of Boonpeng et al. (2018). On the same day as the lichen collection, each lichen thallus was air died overnight (12 h) under a controlled temperature room of approximately 25 ± 2 °C. After that, the lichen samples were dried, cleaned to remove debris and dirt, submerged in deionized water for 2 min, and placed on a plate under ca. 100 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) for at least 2 h and water spraying every 15 min to activate lichen metabolism. Net photosynthesis (Pn) was measured by a portable LI-6400 infrared gas analyzer (IRGA, Li-Cor, Lincoln, NE, USA) by using a conifer chamber in an open system under the following optimal conditions: PAR at 350 µmol m^{-2} s^{-1}, thallus water content ca. 100% air-dry weight, temperature 25 ± 2 °C, air flow rate 500 µmol s^{-1}, and ambient CO_2 (300–400 ppm). Six measurements were performed on each thallus, and an arithmetic mean was calculated. Five thalli (*n* = 5) were measured for each season at each monitoring site.

Chlorophyll fluorescence

The same thallus that finished from the net photosynthetic measurement was immediately transferred to chlorophyll fluorescence measurement, which indicates the efficiency of photosystem II (PSII) (Maxwell and Johnson 2000; Murchie and Lawson 2013). The ΦPSII = (Fm′-F)/Fm′, and ETR (electron transport rate) = ΦPSII × PAR × 0.84 × 0.5 were measured on well–wet samples (sprayed with deionized water) with a MINI-PAM pulse amplitude-modulated fluorometer (Heinz Walz GmbH, Effeltrich, Germany) that applied an actinic light of ca. 350 µmol m^{-2} s^{-1} and a saturating light pulse of ca. 4000 µmol m^{-2} s^{-1} for 0.8 s (Jensen and Kricke 2002) under the controlled temperature room of ca. 25 ± 2 °C. Fv/Fm = (Fm-Fo)/Fm was measured under dark conditions, which were dark-adapted from 1 h before the measurement. Six measurements were performed on each thallus, and an arithmetic mean was calculated. Five thalli (*n* = 5) were measured for each season at each monitoring site.

Photosynthetic pigment extraction

The same thalli that finished the chlorophyll fluorescence measurement were air dried at room temperature for approximately 15 h and weighed at a constant weight. Each thallus was cut into small pieces, weighed, and placed in a test tube. The pigment extraction followed the procedure by Boonpragob (2002), and each sample was washed six times with 3 mL of $CaCO_3$ saturated acetone to remove lichen acids that can intercept light absorbance. Then, residual acetone was allowed to evaporate from the thalli. The sample was immersed in 5 mL dimethyl sulfoxide (DMSO), which contained 2.5 mg/mL polyvinylpolypyrrolidone 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 DMSO was added and left in the dark for ca. 15 h. The optical density of the extract solution was measured at wavelengths of 480, 649, and 665 nm with a spectrophotometer (GENESYS 10S UV−Vis spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) to determine chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (TChl), and total carotenoids (TCar) contents by using the equations by Wellburn (1994). Three measurements were performed on each sample, and an arithmetic mean was calculated. Five samples (*n* = 5) were measured for each season at each monitoring site.

Data analysis

The level of contamination of each element at each monitoring site was estimated using a contamination factor (CF); CF = Cm/Cc, where Cm is the average concentration of an element at a monitoring site and Cc is the average concentration of the same element from all seasons at the control site. Subsequently, a pollution load index (PLI) was calculated using the CF; $PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{1/n}$, where *n* is the number of studied elements. This index was used to assess the air pollution load at each monitoring site (Tomlinson et al. 1980). The level of stress of each physiological parameter at each monitoring site was estimated using a stress factor (SF); SF = Pm/Pc, where Pm is the average value of a physiological parameter at a monitoring site, and Pc is the average value of the same physiological parameter from all seasons at the control site. The overall integrity of lichens at a monitoring site 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 (Boonpeng et al. 2018). The performance of the air for supporting life at each monitoring site was estimated using an air performance index (API); API = (VI/PLI) × 100. This index was slightly modified from the lichen–air quality index (LiAQI = PLI/VI) proposed by our previous study (Boonpeng et al. 2018). The interpretation scales for the CF, PLI, SF, VI, and API are shown in Table 2., where the scales and meanings were slightly modified from (Boonpeng et al. 2018) to provide a clearer interpretation. Pearson's correlation test (*r*) was used to investigate correlations among the elements, physiological parameters, VI, PLI, and API using SigmaPlot 14 (Systat Software Inc., USA).

Results and discussion

Trace element concentrations in the lichen transplants

Concentrations of the elements in the transplanted lichen *P. tinctorum* varied across the monitoring sites and seasons. The highest concentrations of all elements in all seasons were found at schools in the industrial area, while the lowest concentrations of most elements were observed at the control site (Table S2). The element concentrations in the transplanted lichens at the control site also varied across seasons, which most of them were highest in the hot season and lowest in the late rainy season. The average concentration of each element from all seasons at the control site represented

Table 2 Interpretation scales for the contamination factor (CF), pollution load index (PLI), stress factor (SF), vitality index (VI), and air performance index (API)

Scale	Meaning	Indicated color	Scale	Meaning	Indicated color
Contamination Factor (CF)			Stress Factor (SF)		
<1.20	No contamination	Sky blue	>0.95	Normal	Sky blue
1.20-2.00	Light contamination	Light green	0.76-0.95	Light stress	Light green
2.01-3.00	Medium contamination	Yellow	0.51-0.75	Medium stress	Yellow
>3.00	Heavy contamination	Red	≤0.50	Severe stress	Red
Pollution Load Index (PLI)			Vitality Index (VI)		
<1.20	No pollution	Sky blue	>0.95	Healthy	Sky blue
1.20-1.50	Low pollution	Light green	0.76-0.95	Light deterioration	Light green
1.51-2.00	Moderate pollution	Yellow	0.51-0.75	Medium deterioration	Yellow
2.01-2.50	High pollution	Orange	0.26-0.50	High deterioration	Orange
>2.50	Very high pollution	Red	≤0.25	Extreme deterioration	Red
Air Performance Index (API)					
>95	Superb performance	Sky blue			
76-95	High performance	Light green			
51-75	Medium performance	Yellow			
26-50	Low performance	Orange			
0-25	Very low performance	Red			

the average concentration of that element in the 3-month exposed lichen *P. tinctorum* at Khao Yai National Park of all seasons. This value was used to calculate the CF of each element at each monitoring site.

Among the 56 CFs of 14 investigated elements from all seasons at the control site, 50 of them (89%) were grouped as no contamination. The remaining 6 CFs (11%) for Cd, Co, Cr, Cu, Ni, and Sb were in the light contamination group, which was found in the hot and dry seasons (Table 3). These elements probably came from soil resuspension, rock weathering, and decomposition of dead materials. Additionally, the contamination levels of the elements in the schools in the LCB industrial area varied across seasons. The most contaminated period was the hot season (March to May), where 25, 33, 33, and 9% of CFs were classified as heavy, medium, light, and no contamination, respectively. The dry season (December to February) was the second contaminated period, where 19, 34, 27, and 20% of CFs were classified as heavy, medium, light, and no contamination, respectively. The early rainy season (June to August) was the third contaminated period, where 13, 36, 40, and 11% of CFs were classified as heavy, medium, light, and no contamination, respectively. The late rainy season (September to November) was the lowest contaminated season, where 6, 16, 56, and 22% of CFs were classified as heavy, medium, light, and no contamination, respectively.

Among the 14 investigated elements from all seasons, heavy contamination levels were observed for 9 elements, including As, Cd, Co, Cr, Cu, Mo, Pb, Sb, and Ti. Focusing on the elements in the heavy contamination category, Pb had the highest average CF value, followed by Cu, Co, Ti, Cd, Sb, Mo, As, and Cr. These elements may be released by various anthropogenic activities, such as industrial manufacturing, fossil fuel combustion, brake abrasives, clutches, wear of tires, car engines and components, transportation by trucks and ships, household/community activities, cigarette smoke, and natural origins, such as sea aerosols and windblow dust (Adriano 2001; Conti and Cecchetti 2001; Garty 2001; Paoli et al. 2019b; Sorbo et al. 2008).

Irrespective of the emission sources, these elements can adversely affect human health, especially children and elders. The lead at all school sites in all seasons was 3.00 to 10.79 times (5.59 on average) above that at the control site. The average highest average concentration of Pb was observed in the hot season, followed by the early rainy, dry, and late rainy seasons. The highest average concentration of Pb was found at S2, followed by S3, S5, S4, S6, S9, S1, S8, and S7. According to the toxicological profiles ordered by the Agency for Toxic Substances and Disease Registry (ATSDR), this metal was numbered 2 of the ATSDR 2022 substance priority list (ATSDR 2022) and is grouped 2B by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (IARC 2022). It can adversely affect the nervous system, kidney function, immune system, and reproductive, developmental, and cardiovascular systems (ATSDR 2021g). Lead is particularly dangerous to children because their growing bodies absorb more lead than adults, and their brains and nervous systems are more sensitive. The effects of Pb are neurological effects, behavioral problems, learning deficits, and lowered IQ (EPA 2022). Lead in the air can come from burning fossil fuels, metal processing, lead–acid battery manufacturers, and motor vehicles (EPA 2022; Guidotti et al. 2009).

Copper had the second highest contamination level. Its concentrations at all schools ranged from 1.26 to 4.44 times (2.88 on average) above the control site. Most of them were also contaminated in the hot and dry seasons. Copper is an essential element in plants and animals (including humans). This metal was numbered 120 of the ATSDR 2022 substance priority list, and receiving a high concentration can affect the gastrointestinal system. It can originate from manufacturing on wire, bronze pipes, sheet metal, brass, and faucets (ATSDR 2021f), as well as motor vehicles (Zhao et al. 2019).

The concentration of Co at all schools ranged from 1.08 to 4.53 times (2.36 on average) higher than that at the control site, which was mostly observed in the hot and dry seasons. It was numbered 51 of the ATSDR 2022 substance priority

Table 3 The contamination factors (CFs) and pollution load indices (PLIs) of the elements, the stress factors (SFs) and vitality indices (VIs) of the physiological parameters, and the air performance indices (APIs) of the lichen Parmotrema tinctorum exposed in the dry, hot, early rainy, and late rainy seasons at schools in the industrial area at Laem Chabang municipality (S1–S9) and at the control site in Khao Yai National Park (C)

Site	CF														PLI	SF								VI	API
	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Ti	V	Zn		Pn	Fv/Fm	ΦPSII	ETR	Chl a	Chl b	TChl.	TCar.		
Dry season																									
C-KY	0.79	0.72	1.46	0.83	1.15	0.74	1.04	0.82	1.02	1.07	0.48	1.03	1.17	0.98	0.92	0.87	1.00	0.85	0.85	0.79	0.82	0.80	0.89	0.86	93
S1-WNK	1.83	2.37	1.77	2.02	3.57	1.17	1.05	2.41	1.39	3.78	1.92	2.32	2.59	1.12	1.95	0.52	0.89	1.04	1.04	0.81	0.77	0.80	0.91	0.83	43
S2-WPB	1.88	1.18	4.53	2.27	3.31	2.57	1.43	2.32	0.32	9.24	1.28	3.08	2.87	1.15	2.06	0.52	0.88	1.04	1.04	0.96	0.93	0.96	1.12	0.91	44
S3-WPP	2.92	2.71	4.46	2.17	3.87	1.16	1.42	3.32	1.95	6.39	2.08	2.13	2.89	1.18	2.46	0.58	0.80	0.79	0.79	0.74	0.78	0.75	0.92	0.76	31
S4-BBL	1.84	2.10	2.73	1.43	2.86	1.29	1.39	1.64	0.92	4.56	1.44	2.34	2.52	1.06	1.83	0.49	0.77	0.75	0.75	0.85	0.86	0.85	0.97	0.77	42
S5-WMN	2.19	4.09	2.47	2.88	3.56	1.42	1.26	1.82	0.98	6.06	1.65	1.47	2.55	1.19	2.10	0.00	0.04	0.05	0.05	0.19	0.35	0.23	0.39	0.06	N/A
S6-WLC	2.32	2.40	1.29	2.83	3.61	1.15	0.68	1.95	0.93	4.58	2.03	2.36	2.03	0.80	1.80	0.00	0.03	0.01	0.01	0.11	0.41	0.18	0.31	0.03	N/A
S7-WBN	2.70	3.98	2.87	3.12	1.94	1.89	1.59	2.77	1.57	3.59	0.80	2.76	2.76	1.14	2.20	0.00	0.56	0.33	0.33	0.56	0.73	0.60	0.71	0.22	10
S8-WMR	2.70	5.38	3.23	2.86	4.03	1.57	1.37	2.68	1.25	4.67	2.72	2.38	2.55	1.07	2.48	0.00	0.45	0.30	0.30	0.46	0.56	0.48	0.71	0.19	8
S9-LCM	1.90	1.29	1.29	1.09	1.69	1.07	1.19	2.32	0.86	4.55	1.12	2.15	1.85	0.72	1.47	0.00	0.38	0.27	0.27	0.54	0.64	0.57	0.70	0.19	13
Hot season																									
C-KY	1.06	1.27	1.15	1.67	1.25	1.06	1.02	1.14	1.30	1.03	1.49	0.88	1.01	0.99	1.15	0.95	1.03	1.19	1.18	1.06	1.06	1.06	0.95	1.06	92
S1-WNK	1.81	3.41	1.53	2.41	3.70	1.38	1.12	2.59	1.50	5.12	2.35	2.64	2.51	1.21	2.16	0.66	0.98	1.15	1.15	1.20	1.19	1.19	1.18	1.07	N/A
S2-WPB	1.92	1.83	4.36	2.06	3.57	2.87	1.50	2.32	0.54	10.79	2.13	3.17	2.73	1.30	2.35	0.94	0.96	1.40	1.39	1.03	1.02	1.03	1.04	1.09	N/A
S3-WPP	2.50	3.48	3.76	1.98	4.44	1.32	1.74	3.45	2.15	8.00	2.45	1.87	2.90	1.27	2.61	0.44	0.98	1.14	1.13	0.99	1.02	1.00	1.06	0.94	N/A
S4-BBL	1.66	2.21	2.99	1.82	3.66	1.48	1.41	1.91	1.19	6.81	1.39	2.92	2.91	1.11	2.09	0.48	0.99	1.22	1.20	1.07	1.03	1.06	1.08	0.98	N/A
S5-WMN	2.32	3.62	3.45	2.60	4.07	1.62	1.79	2.00	1.46	6.75	2.56	1.86	2.68	1.20	2.44	0.00	0.31	0.34	0.33	0.13	0.47	0.21	0.38	0.13	N/A
S6-WLC	2.65	2.85	1.72	2.83	4.07	1.17	0.84	2.45	1.50	6.35	2.61	2.54	2.29	0.93	2.16	0.00	0.00	0.00	0.00	0.08	0.47	0.17	0.29	0.01	N/A
S7-WBN	2.48	3.80	3.38	2.19	2.85	1.94	1.64	3.27	1.82	3.96	1.23	3.26	2.82	1.16	2.39	0.58	0.97	1.39	1.39	0.98	1.11	1.01	1.08	1.03	N/A
S8-WMR	3.60	4.84	3.83	2.80	4.15	1.87	1.67	2.68	1.44	4.71	2.88	2.92	2.60	1.13	2.70	0.08	0.92	0.98	0.98	1.02	1.09	1.03	1.13	0.75	N/A
S9-LCM	1.88	2.10	1.46	1.93	2.57	1.53	1.30	3.00	1.03	4.94	1.39	2.17	1.94	0.96	1.83	0.39	0.97	1.22	1.22	1.09	1.08	1.08	1.10	0.97	N/A
Early rainy season																									
C-KY	1.10	1.11	0.77	0.83	0.88	1.16	1.11	1.14	0.85	1.02	1.12	1.08	0.92	1.04	1.00	0.82	1.03	0.92	0.92	1.28	1.15	1.25	1.20	1.06	106
S1-WNK	1.37	1.36	1.41	1.91	2.12	1.32	1.18	1.59	1.42	5.89	3.57	2.61	2.44	1.19	1.86	0.48	0.96	0.93	0.92	1.05	0.96	1.03	1.03	0.90	48
S2-WPB	1.75	0.95	1.84	1.16	2.79	2.99	1.43	1.68	0.36	6.77	2.45	2.50	2.43	1.32	1.79	0.21	0.91	0.91	0.91	1.11	1.02	1.09	1.13	0.83	46
S3-WPP	1.75	2.17	2.63	1.96	2.76	1.67	1.70	2.36	1.75	7.74	3.79	2.09	2.53	1.32	2.32	0.05	0.94	1.00	1.00	0.95	0.93	0.95	0.99	0.66	29
S4-BBL	1.84	1.58	2.49	1.01	2.73	1.61	1.45	1.59	1.07	6.75	3.68	2.63	2.57	1.13	1.99	0.45	0.93	0.98	0.98	1.08	1.02	1.07	1.01	0.91	46
S5-WMN	1.63	1.97	2.85	1.85	3.11	2.20	1.53	1.27	1.16	6.71	4.59	1.93	2.35	1.19	2.14	0.22	0.93	0.98	0.96	1.05	0.99	1.04	1.04	0.83	N/A
S6-WLC	1.48	2.37	1.80	1.82	3.30	2.29	0.83	2.41	1.36	6.34	3.09	2.63	2.20	1.16	2.09	0.00	0.54	0.42	0.42	0.80	0.96	0.84	0.86	0.26	N/A
S7-WBN	2.16	2.06	2.40	1.52	1.84	2.13	1.63	2.41	1.39	3.42	2.56	2.62	2.63	1.40	2.08	0.39	0.96	1.19	1.19	0.95	0.93	0.94	0.95	0.90	43
S8-WMR	2.10	2.71	2.73	1.46	2.73	1.98	1.75	2.09	1.45	5.35	3.47	2.72	2.62	1.22	2.28	0.16	0.86	1.04	1.04	1.19	1.23	1.20	1.19	0.86	38
S9-LCM	2.01	1.92	1.84	1.99	1.54	1.98	1.56	2.05	1.15	5.50	2.03	2.26	2.00	1.17	1.92	0.41	0.91	1.00	1.00	0.96	0.91	0.95	0.97	0.86	45
Late rainy season																									
C-KY	1.06	0.90	0.62	0.67	0.73	1.04	0.83	0.91	0.84	0.87	0.91	1.01	0.91	1.00	0.87	1.36	0.94	1.05	1.05	0.87	0.97	0.89	0.96	1.00	115
S1-WNK	1.11	1.22	1.44	1.54	2.00	1.26	0.83	1.32	1.25	4.50	2.45	1.73	1.68	0.93	1.51	0.54	0.78	0.67	0.67	1.12	0.99	1.09	1.14	0.84	56
S2-WPB	1.33	1.24	1.10	1.21	2.34	2.51	1.25	1.09	0.30	4.25	1.92	2.12	1.63	1.15	1.45	0.59	0.72	0.64	0.64	1.12	1.13	1.12	1.16	0.85	59
S3-WPP	1.26	1.56	1.96	1.42	2.39	1.44	1.40	1.41	1.16	5.96	2.67	1.82	2.00	1.12	1.76	0.43	0.67	0.62	0.62	0.94	0.89	0.93	0.95	0.73	41
S4-BBL	1.57	1.18	1.60	0.93	2.24	1.52	1.19	1.27	0.97	4.43	2.29	2.15	2.26	0.89	1.58	0.32	0.71	0.68	0.68	1.02	0.93	1.00	1.07	0.76	48
S5-WMN	1.28	1.24	1.94	1.40	2.71	1.82	1.21	1.36	1.08	5.36	2.88	1.47	1.63	0.82	1.65	0.16	0.48	0.42	0.42	0.98	0.91	0.96	1.03	0.58	N/A
S6-WLC	1.02	1.65	1.08	1.61	2.51	1.81	0.60	1.55	0.84	4.94	2.72	2.14	1.77	0.87	1.55	0.19	0.66	0.52	0.52	1.21	1.15	1.20	1.25	0.72	N/A
S7-WBN	1.86	1.85	1.56	1.23	1.40	2.11	1.20	1.68	1.15	3.00	1.92	1.97	1.67	1.20	1.64	0.43	0.78	0.52	0.52	1.00	0.91	0.98	1.07	0.74	45
S8-WMR	1.35	1.45	1.72	1.14	2.25	1.55	1.20	1.55	1.14	4.39	2.19	2.10	1.85	0.88	1.63	0.21	0.70	0.69	0.69	0.86	0.81	0.85	0.97	0.67	41
S9-LCM	1.35	1.51	1.37	1.49	1.28	1.80	1.07	1.50	1.04	5.19	1.33	1.98	1.36	0.95	1.50	0.40	0.79	0.64	0.64	0.95	0.85	0.93	0.98	0.75	50
Average all seasons																									
C-KY	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	100
S1-WNK	1.53	2.09	1.54	1.97	2.85	1.28	1.05	1.98	1.39	4.83	2.57	2.32	2.30	1.11	1.89	0.55	0.90	0.95	0.94	1.04	0.98	1.03	1.07	0.92	49
S2-WPB	1.72	1.30	2.96	1.68	3.00	2.74	1.40	1.85	0.38	7.76	1.95	2.72	2.41	1.23	1.94	0.56	0.87	1.00	0.99	1.06	1.03	1.05	1.11	0.94	50
S3-WPP	2.11	2.48	3.20	1.88	3.36	1.40	1.57	2.64	1.75	7.02	2.75	1.98	2.58	1.22	2.32	0.37	0.85	0.89	0.89	0.90	0.91	0.91	0.98	0.81	34
S4-BBL	1.73	1.77	2.46	1.30	2.87	1.47	1.36	1.60	1.04	5.64	2.20	2.51	2.56	1.05	1.90	0.43	0.85	0.91	0.90	1.01	0.96	1.00	1.03	0.86	45
S5-WMN	1.85	2.73	2.68	2.18	3.36	1.77	1.45	1.61	1.17	6.22	2.92	1.68	2.30	1.10	2.12	0.00	0.44	0.44	0.44	0.59	0.68	0.61	0.71	0.22	N/A
S6-WLC	1.87	2.32	1.47	2.27	3.37	1.61	0.74	2.09	1.16	5.55	2.61	2.42	2.07	0.94	1.92	0.00	0.31	0.24	0.24	0.55	0.75	0.60	0.68	0.18	N/A
S7-WBN	2.30	2.92	2.55	2.01	2.01	2.02	1.52	2.53	1.48	3.49	1.63	2.65	2.47	1.23	2.12	0.33	0.82	0.86	0.86	0.87	0.92	0.88	0.95	0.78	33
S8-WMR	2.44	3.59	2.88	2.06	3.29	1.75	1.50	2.25	1.32	4.78	2.81	2.53	2.41	1.08	2.30	0.03	0.73	0.75	0.75	0.88	0.92	0.89	1.00	0.56	29
S9-LCM	1.79	1.71	1.49	1.63	1.77	1.60	1.28	2.22	1.02	5.05	1.47	2.14	1.79	0.95	1.70	0.24	0.76	0.78	0.78	0.89	0.87	0.88	0.94	0.72	36

The net photosynthesis (Pn) that showed negative values and the chlorophyll fluorescence (Fv/Fm, ΦPSII, and ETR) that showed the value of 0.00 replaced the values by 0.0001 before the calculation of the SFs. The colors indicated categories of the CF, PLI, SF, VI, and API according to the Table 2. The APIs at S5 and S6 in all seasons and the values in the hot season were not calculated because the VIs in this season need more studies. The APIs of average all seasons at all industrial sites were calculated excluding the values from the hot season

list. It can affect cardiovascular, hematological, and respiratory systems (ATSDR 2021e), and cobalt(II) oxide is possibly carcinogenic to humans (IARC 2022). Cobalt compounds are used to color glass, and ceramics and paints and are used as a drier for porcelain enamel and paints (ATSDR 2021e).

The concentration of Ti at all schools ranged from 1.47 to 3.26 times (2.33 on average) above the control site. Titanium tetrachloride can affect the respiratory system (ATSDR 2021i). It is used to make titanium metal and other titanium-containing compounds, such as titanium dioxide, which is used as a white pigment in paints and other products and to produce other chemicals.

The concentration of Cd at all schools ranged from 0.95 to 5.38 times (2.32 on average) above the control site. It was numbered 7 of the ATSDR 2022 substance priority list. It can affect cardiovascular, developmental, gastrointestinal, neurological, renal, reproductive, and respiratory systems (ATSDR 2021c) and is in Group 1 of IARC as carcinogenic to humans (IARC 2022). This element can originate from the manufacturing of batteries, pigments, metal coatings, and plastics (ATSDR 2021c) and by motor vehicles (Zhao et al. 2019).

The concentration of Sb at all schools ranged from 0.88 to 4.59 times (2.32 on average) above the control site. It was numbered 236 of the ATSDR 2022 substance priority list. It can affect cardiovascular and respiratory systems, and trivalent antimony is probably carcinogenic to humans (ATSDR 2021a). This element is used in lead storage batteries, solder, sheet and pipe metal, textiles, plastics, paints, ceramics, and as enamels for plastics, metal, and glass (ATSDR 2021a).

The concentration of Mo at all schools ranged from 1.09 to 3.45 times (2.09 on average) above the control site. It can affect reproductive and respiratory systems (ATSDR 2021h), and molybdenum trioxide is possibly carcinogenic to humans (IARC 2022). Molybdenum is used widely in industry to make steel alloys (ATSDR 2021h).

The concentration of As at all schools ranged from 1.02 to 3.60 times (1.93 on average) above the control site. It was numbered 1 of the ATSDR 2022 substance priority list. It can affect dermal, gastrointestinal, hepatic, neurological, and respiratory systems (ATSDR 2021b) and is the Group 1 IARC that is carcinogenic to humans (IARC 2022). Inorganic arsenic compounds are mainly used to preserve wood, and organic arsenic compounds are used as pesticides (ATSDR 2021b).

The concentration of Cr at the industrial sites ranged from 0.93 to 3.12 times (1.89 on average) above the control site. It was numbered 78 of the ATSDR 2022 substance priority list. It can affect immunological, renal, and respiratory systems (ATSDR 2021d), and chromium(VI) compounds are carcinogenic to humans (IARC 2022). It is used for making steel, chrome plating, dyes and pigments, leather tanning, and wood preservation (ATSDR 2021d).

In addition, the concentration of V at all schools ranged from 1.36 to 2.91 times (2.32 on average) above the control site. Although it did not show heavy contamination at any monitoring site or season, its average concentration was remarkably high along Cd, Sb, and Ti. It was numbered 208 of the ATSDR 2022 substance priority list. It can affect cardiovascular, gastrointestinal, renal, reproductive, and respiratory systems (ATSDR 2021j), and vanadium pentoxide is possibly carcinogenic to humans (IARC 2022). This element can come from oil refineries and steel factories and is used for automobile parts (ATSDR 2021j).

Not only the concentrations and toxic prioritization, the toxicity of the above-mentioned elements also depends on the exposed time, age, and health of receivers. In addition, activity such as walking, running, and playing sports increase uptake of air pollutants, as well as commute to schools, including walking, bicycles, motorcycles, cars, vans, and buses, also a factor of uptalking air pollutants. Previous studies reported that the concentrations of Co, Cu, Fe, Mn, Ni, Pb, and Zn in the lichens *Ramalina celastri* and *Usnea amblyoclada* were correlated with pharyngitis, tonsilitis, asthma, laryngitis, and allergic rhinitis in children under six years old (Carreras et al. 2009). Additionally, the concentration of Co in the lichen *Canoparmelia texana* was linked with cardiovascular diseases in adults (Saiki et al. 2014).

Concentrations of the study elements in the lichens at each school can originate from several sources, including road traffic, ships, port activities, industrial factories, petrochemical plants, wind-blown dust, and sea spray. Overall air pollution loads at each monitoring site and season based on the 14 investigated elements were illustrated by the PLIs (Table 3 and Fig. 2 a). The PLIs at all schools in all seasons were clearly higher than those at the control site, indicating higher air pollution. At each school, the pollution loads varied across seasons. The highest PLIs at all schools were observed in the hot season, and the lowest PLIs were in the late rainy season, except for S9, where the highest PLI was discovered in the early rainy season. The PLIs in the dry season were comparable to those in the early rainy seasons. In the hot season, the PLIs at all schools ranged from 1.83 (S9) to 2.70 (S8) (2.30 on average) and were categorized as moderate to very high pollution. Two schools, including S3 and S8, had very high pollution, six schools, including S1, S2, S4, S5, S6, and S7, had high pollution, and one school (S9) had moderate pollution. In this period, medium rainfall and the prevailing wind blew from the southwest; therefore, most of the schools were downwind of the industrial areas, especially S8, which was close to the major roads and the industrial area. Additionally, S3 was adjacent to busy roads by trucks and had fewer trees to prevent the diffusion of air pollutants from the road. This indicates that road traffic was another important source of the elements. In the dry season, the PLIs at all schools ranged from 1.47 (9) to 2.48 (S8) (2.04 on average) and were categorized as low pollution to high pollution. Five sites had high pollution (S2, S3, S5, S7, and S8), three sites had moderate pollution (S1, S4, and S6), and one other site had low pollution (S9). This season had higher PM10, PM2.5, and O_3 concentrations and the lowest rain amount compared to the other seasons. Most of the wind in this season came from the east; therefore, S9 was upwind of the major roads and the industrial area and therefore had lower air pollution. The other sites, especially S8, S3, S7, S5, and S2, were still downwind of the major roads or the industrial area. In the early rainy season, the PLIs at all schools ranged from 1.79 (S2) to 2.32 (S3) (2.05 on average) and were categorized as moderate to high pollution. Five sites had high pollution (S3, S5, S6, S7, and S8), and the other 4 sites had moderate pollution (S1, S2, S4, and S9). The prevailing wind was also from the southwest but had more rain than the hot season. This might be the reason why it had lower pollution in comparison with the hot season. Last, in the late rainy season, the PLIs at all schools ranged from 1.45 (S2) to 1.65 (S5) (1.59 on average) and were categorized as low pollution (S2, S9) to medium pollution (S1, S3, S4, S5, S6, S7, and S8). The prevailing winds equally came from the southwest and the east. This season had heavy rain at the beginning of the period that probably cleaned the air and resulted in better air quality compared to the other seasons. The average PLIs in all seasons at each site can be ranked in the following descending order: S3 > S8 > S5 > S7 > S2 > S6 > S4 > S1 > S9 > C. S9 was in the residential area of the LCB downtown. Although it had the shortest distance to the center of the LCBIE, it was surrounded by the residential area that probably was a barrier of

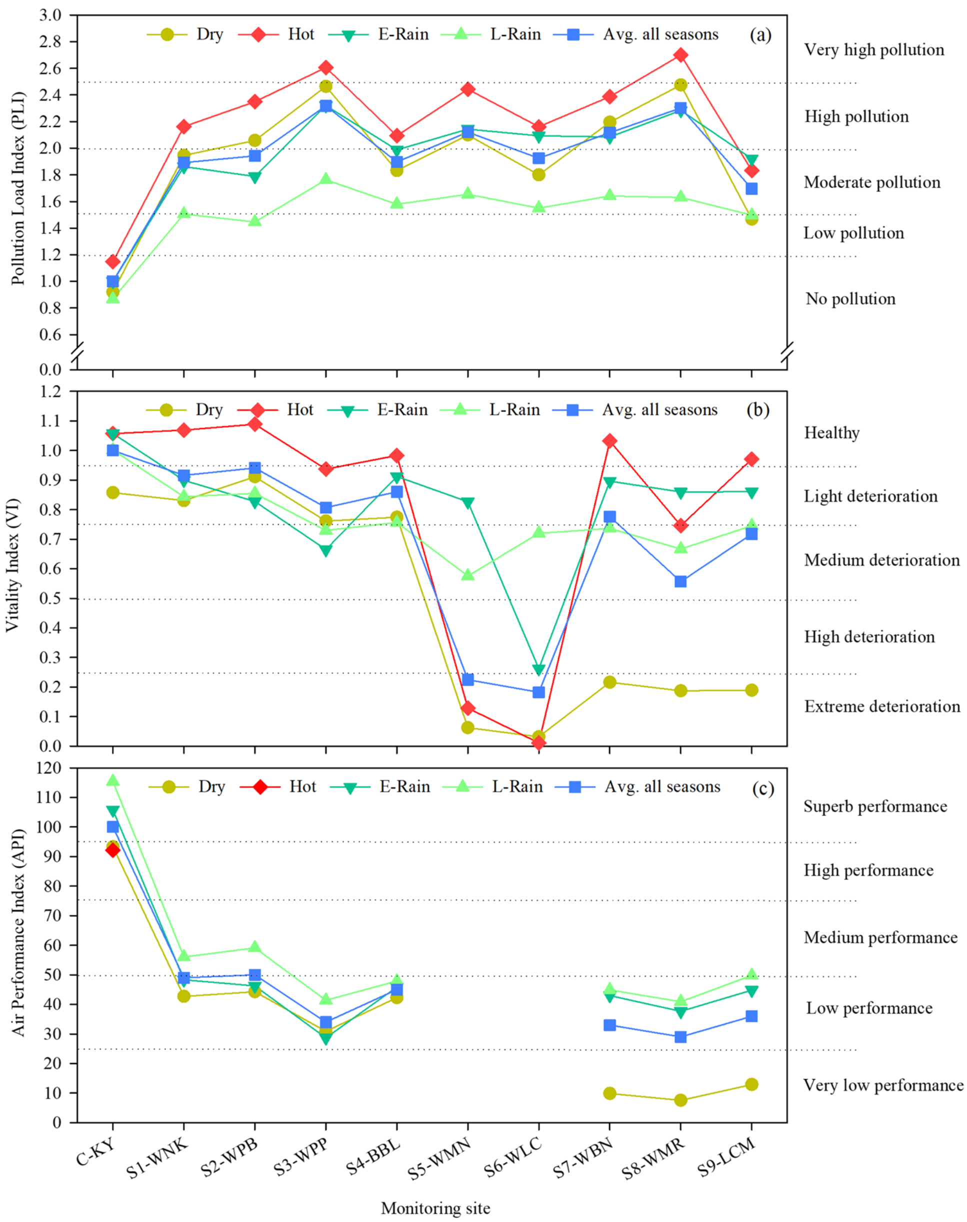

Fig. 2 The pollution load indices (PLIs, **a**), vitality indices (VIs, **b**), and air performance indices (APIs, **c**) of the lichen *Parmotrema tinctorum* exposed in the dry, hot, early rainy (E-Rain), and late rainy (L-Rain) seasons, as well as the average from all seasons (Avg. all seasons) at schools in the industrial area in Laem Chabang municipality (S1–S9) and at the control site in Khao Yai National Park (C). The categories of each index were defined according to Table 2.. The APIs at S5 and S6 in all seasons and the values in the hot season were not calculated because the VIs in this season need more study. The APIs of the average of all seasons at all industrial sites were calculated excluding the values from the hot season

the elements from the major roads and the industrial plants. Additionally, S1 was located farthest from the LCBIE center but approximately 1.5–2 km from the highway (route 7). S3 and S8 had the highest PLIs compared to the other sites, and they were close to the major and busy roads by trucks and industrial factories. Based on the distance from the center of the LCBIE, air pollution in all three zones in the industrial area was comparable in the hot and dry seasons, while in the two rainy seasons, lower air pollution was clearly observed in the outer zone (Fig. 3 a).

Irrespective of the emission sources of the elements, these elements can adversely affect human health, especially children. Therefore, improving school environments should be an urgent issue for all the studied schools, particularly those adjacent to the major and busy roads and industrial plants, such as S3 and S8. Many studies in the literature have confirmed that proximity to road traffic is the main factor of poor air quality at schools (An et al. 2021; Osborne et al. 2021a, 2021b; Requia et al. 2021a, 2021b). Poor air quality was reported at schools in industrial areas (Al-Hemoud et al. 2017; Godoi et al. 2013; Villanueva et al. 2018), as well as at schools in urban areas (Canha et al. 2012, 2014; Paoli et al. 2019a; Protano et al. 2017). Increasing greenspace in schools and building green walls/green barriers in front of schools and along roads can reduce air pollutants and create a more livable environment at schools (McDonald et al. 2016; Nowak et al. 2006; Osborne et al. 2021a).

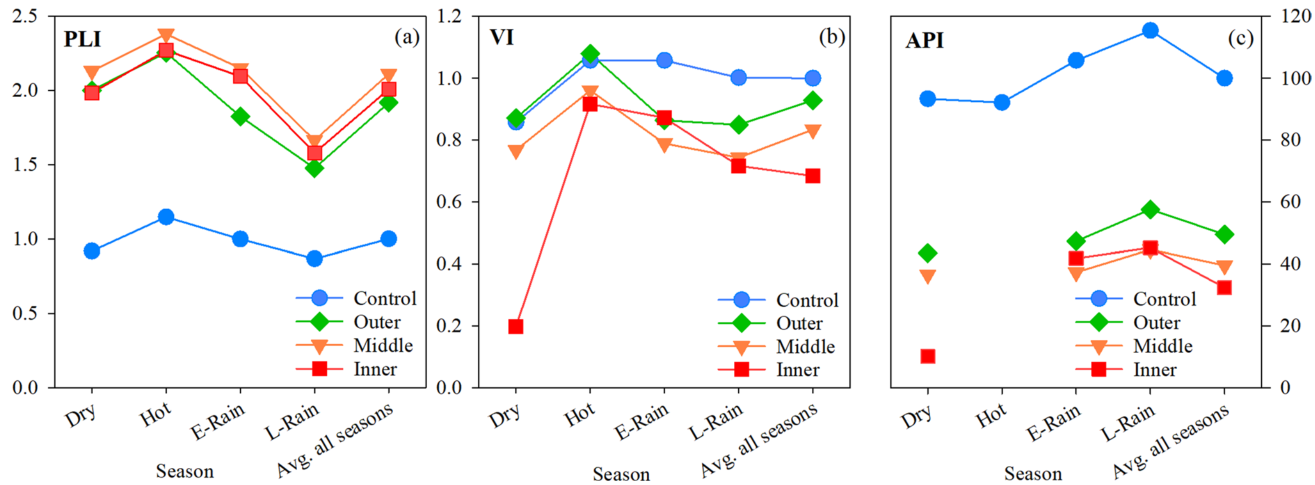

Fig. 3 The pollution load indices (PLIs, **a**), vitality indices (VIs, **b**), and air performance indices (APIs, **c**) of the lichen *Parmotrema tinctorum* exposed in the dry, hot, early rainy (E-Rain) and late rainy (L-Rain) seasons, as well as the average from all seasons (Avg. all seasons) in each zone based on the distance from the center of the Laem Chabang industrial estate, including the inner zone (0–3 km; S7, S8, and S9), the middle zone (3–6 km, S3 and S4), the outer zone (6–9 km, S1 and S2), and the control site (156 km, C). The average APIs in the middle and outer zones did not include S5 and S6, and the values in the hot season were not calculated because the VIs in this season need more study

Physiological responses of the lichen transplants

Lichens are among the most sensitive species to air pollution (Nash III 2008b). Pollutants primarily affect the physiology of lichens, followed by their morphology, population, and community. Therefore, physiological alterations in lichens can be used as an early warning sign of environmental stress caused by air pollution (Daimari et al. 2021; Malaspina et al. 2018; Paoli et al. 2015; Sujetovienė et al. 2020). The change in the physiological parameters of lichens is the result of overall air pollution in the study area, not from a single pollutant, and it can have synergistic and/or additive effects. Therefore, the study of physiological responses is very useful for estimating overall air quality, while the study of element concentrations provides information on only the investigated elements. The physiological parameters of the transplanted lichen *P. tinctorum* responded depending on the monitoring sites and seasons. The lowest values of all elements in all seasons were discovered at schools in the industrial area (Table S3). The average values of each parameter from all seasons at the control site were used to calculate the SF of each parameter at each site. Among the 32 SFs of 8 physiological parameters from all seasons at the control site, 17 (53%) SFs were normal, and 15 (47%) SFs were light stress (Table 3). Among the light stress categories, 47% were in the dry season, indicating that climatic conditions, especially lower air humidity, were an important factor influencing the physiological parameters of the lichens. Due to the different climatic conditions at the schools in the industrial area and the control site, it is better to use the physiological parameters at a local/internal control site that has similar climatic conditions for the comparison (Lucadamo et al. 2015). S1 and S2 were suitable for use as local control sites because their VI values in all seasons were closest to the VIs of the control site, especially in the dry and hot seasons, where they were in the same categories (Table 3 and Figs. 2 b and 3 b). A total of 288 SFs at all schools in all seasons were categorized as normal (41%), light stress (19%), medium stress (15%), and severe stress (25%). Most of the severe stress of the SFs occurred in the dry season (42%), followed by the hot (28%), early rainy (16%), and late rainy seasons (14%). This indicates that the dry season had worse air quality than the other seasons. Considering the studied physiological parameters, net photosynthesis (Pn) was the most sensitive parameter, followed by chlorophyll fluorescence parameters (Fv/Fm > ΦPSII, ETR), chlorophyll contents (Chl a, TChl > Chl b), and total carotenoids (TCar). This was consistent with results found in the lichen *Ramalina lacera* transplanted in the industrial region in Israel (Garty et al. 2001).

The overall lichen vitality at each monitoring site was estimated using the VIs (Table 3 and Fig. 2 b). The VIs at S5 and S6 showed extremely low values, especially in the dry and hot seasons. The morphology of the lichen thalli of these two sites was also extremely deteriorated (Fig. S5). Morphological damage was investigated, including discoloration (pinkish or brownish), bleaching (death of the algal cells) and necrosis. This morphological change can be used as an indicator for monitoring the early biological effects of air pollution (Gries 1986; Rangel-Osornio et al. 2021). These sites were located close to the industrial area and the industrial port, which could possibly receive higher air pollutants, and black carbon was clearly observed on the lichen thalli. However, these sites were also located close to the sea (100–200 m away), which might also be affected by sea salt. Chowaniec and Rola (2022) observed that salt solutions can decrease the Fv/Fm of the lichens *Hypogymnia physodes* and *Pseudevernia furfuracea*. Therefore, the worsening lichens at these two sites were not guaranteed to be caused by air pollutants alone or the combination of air pollutants and sea salt. For this reason, more studies are needed to elucidate air pollution at these two sites, and at this time, it should be considered from the PLIs. The average VIs in each season were calculated from all schools, except S5 and S6. The highest average VI was observed in the hot season, where the VIs of most sites were classified as healthy. The second highest average VI was found in the early rainy season, where most sites were classified as light deterioration. The third highest average VI was the late rainy season, where most sites were classified as having medium deterioration. Last, the dry season had the lowest average VIs, where 3 sites (excluding S5 and S6) were classified as having severe deterioration. This result was in accordance with the average PM10, PM2.5, and O_3 that showed the highest concentrations in the dry season (Fig. S4, Table S1). This indicates that the VIs of lichens can reflect air quality similar to the air quality monitoring station. The higher lichen vitalities at most sites in the hot season were not clear and need more study. Most likely, the first rain of the season may bring fertilizers from the air to accumulate in the lichen and promote chlorophyll contents, carotenoids, and the efficiency of photosystem II (PSII), which is estimated by the chlorophyll fluorescence parameters. If so, VIs of the lichens in the industrial sites in the hot season should not be used to calculate the APIs. The VIs at sites in the inner and middle zones were clearly lower than those in the outer zone, according to the PLIs at sites in the inner and middle zones, which had higher values than those in the outer zone, indicating that there was poorer air quality (Fig. 3 a and b).

The VIs and PLIs had no correlation; in addition, most elements did not show a correlation with the physiological parameters (Table S4). This indicates that the 14 investigated elements were not the main pollutants that affected the physiology of the lichens. There are several kinds of air pollutants that can affect lichen vitality and human health, such as PAHs, VOCs, POPs, NO_2, SO_2, and PM. Therefore, it is not surprising that the responses of PLIs and VIs at the monitoring sites and seasons showed different trends. The PLIs reflect the pollution load based on the studied elements, while the VIs reflect the effects of overall air pollution.

Air performance index (API) and air quality at the schools

The API derived from the integration between PLI and VI. This index could be useful for air quality assessment because it is based on the investigation of pollutants and their effects on lichens. The APIs of this study were calculated based on the 14 investigated elements and 8 physiological parameters in the lichen *P. tinctorum* (Table 3 and Fig. 2 c). It was negatively correlated with the PLIs ($r = -0.40$, $p < 0.05$) and was positively correlated with the VIs ($r = 0.92$, $p < 0.001$). The APIs at the schools in the industrial area in all seasons were categorized as medium to very low performance, which most of them belonged to the low performance. Based on the obtained API values, the air quality at the monitoring sites varied across seasons. Most sites had the best air performances in the late rainy season, while the worst air performances were observed in the dry season, which was consistent with the data from the air quality monitoring station. Excluding S5 and S6, the average APIs at all schools in all seasons (except the hot season) ranged in descending order: S2 > S1 > S4 > S9 > S3 > S7 > S8. The inner and middle zones clearly showed lower air quality than the outer and control zones (Fig. 3 c).

The results of this study indicated that all selected schools were polluted by several elements, especially in the dry and hot seasons. Children are among the most sensitive groups to air pollution (Makri and Stilianakis 2008); therefore, environmental quality around schools should be improved for better health, life, and welfare and to maintain their academic performance (Lu et al. 2021; Rojas-Vallejos et al. 2021). Lu et al. (2021) found that increase in PM2.5 concentrations was associated with a 0.007 lower average math test scores, and a 0.004 lower average English language/arts test scores of US children. Similar associations were observed for NO_2 and O_3 on math and for NO_2 on English language/arts, while Requia et al. (2021a) reported that proximity of schools to roads affected students' academic performance in Brazil. They found that the highest effects occur in the first buffer, with 250 m. According to our research, the schools that were located close to the major and busy roads and industrial plants such as S3, S7, and S8 (including S5 and S6) should increase greenspace and build green walls/green barriers to intercept and remove air pollutants. In addition, green barriers and suitable trees should be grown along the road network to mitigate or prevent air pollutants from the roads to the surrounding areas. This study clearly showed that the studied schools were contaminated by several airborne trace elements. However, there are many air pollutants emitted from such industrial complexes and road traffic, such as NO_2, SO_2, PM, O_3, VOCs, PAHs, and POPs; therefore, there is a need for more studies on air quality in this area. PM2.5 and PM10 are of particular concern, although their concentrations at most times of the year were below the national standards, but they were above the 2021 WHO guidelines (WHO 2021) at most times of the year, especially PM2.5 (Fig. S4).

Conclusions

Air quality at nine schools in the industrial area at LCB can be assessed by the transplanted lichen *P. tinctorum*. The concentrations of 14 investigated elements were clearly higher at the schools than at the control site. As suggested by the CFs, 9 out of the 14 elements, including As, Cd, Co, Cr, Cu, Mo, Pb, Sb, and Ti, were heavily contaminated in the school environments, especially Pb, which was 3 to 11 times higher than the control site. These elements can originate from various sources, including industrial plants, transportation, road traffic, and ships. The most polluted time by the investigated elements was the hot season (March to May), and the lowest pollution was the late rainy season (September to November). The PLIs indicated that schools in the inner and middle zones clearly had higher pollution loads than the schools in the outer zone during the rainy seasons, while the hot and dry seasons showed similar pollution levels in all zones. Road traffic was a major source of elements at schools in the outer zone. The physiological parameters in the lichens demonstrated the effects of overall air pollution in the environment at each site and can be used as effective early warning signs of air pollution. As illustrated by the VIs, the lichen vitalities estimated based on 8 studied physiological parameters revealed that the lower lichen vitalities at most schools were observed in the dry season and at the schools in the inner and middle zones. Air quality at each school was preliminarily assessed based on the investigated elements and the physiological parameters expressed as the API values. The APIs revealed that poorer air quality at most schools was found in the dry season and at the schools in the inner and middle zones in comparison to the schools in the outer zone. This study clearly showed that the transplanted lichen *P. tinctorum* was an effective bioindicator of air quality in school environments. The results of this study illustrated that all the studied schools were contaminated by air pollutants and that children are one of the most vulnerable groups to air pollution, which can affect their health and academic performance. Therefore, improving air quality at schools is crucial and should be an urgent issue for maintaining good health and may benefit children's academic achievements and careers in the long run. Greenspace, green walls, green barriers, and suitable trees along roads should intercept/prevent/mitigate/remove air pollutants. Additional research on air quality in this area is needed, and future studies should be focused on PM, VOCs, and PAHs. In particular, PM2.5 showed concentrations above WHO guidelines at most times of the year. The results of this study could be useful for policymakers, urban planners, and school design for improving school air quality.

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Data Availability All data generated or analyzed during this study are included in this published article, the supplementary information, and the raw data are available from the corresponding author on reasonable request.

Declarations

Ethical approval This is not applicable.

Consent to participate This is not applicable.

Consent to publication This is not applicable.

Competing interests The authors declare no competing interests.

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