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Carbon Monoxide and its Relation to Species Richness and Density of Epiphytic Terrestrial Microalgae

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Abstract: Some microalgal species are known to be able to tolerate the presence of atmospheric pollutants. However, this is at the expense of other species. Some species are pollutant tolerant while others are pollutant intolerant. Thus, this study is conducted to understand the relationship between Carbon monoxide (CO) and epiphytic terrestrial algae. The samples of algae was collected from four sites namely Rawang and Pelabuhan Kelang to represent the urban area while Kuala Selangor and Port Dickson represent the rural area. All sites sustained approximately >10 species of algae. For data feasibility, only algae with density of more than 30×10^4 cells per cm^2 are being counted. Results showed that the urban area recorded slightly higher species richness (4 species) compared to the rural area (3 species). The urban sites which recorded higher concentration of carbon monoxide (11 μ gm^{-3}) compared to the rural sites (2 μ gm^{-3}), support the growth of Desmococcus olivaceus up to 6-fold with algal density of 12390×10^4 cells/cm^2. In the rural area, Desmococcus olivaceus also dominating the area with much lower density at 2985×10^4cell/cm^2. Another algal species that inhabit the urban site is Trebouxia arboricola which also recorded high density at 12120×10^4cells per cm^2. Jaccard Similarity Index showed only 40% similarity between sites of low and high CO concentration. The cosmopolitan species in this study is Trentepohlia umbrina. However, the density of this species belongs to the lower range compared to other species at only 30×10^4 cell/cm^2. The results also showed that the concentration of carbon monoxide and algal density are positively correlated. Carbon monoxide showed a strong positive correlation (R = 0.901). This research concludes that the urban area which contained higher CO ultimately increase the density of algae compared to the rural area.

Key words: Carbon monoxide, algae, air pollution, species richness, density

INTRODUCTION

Algae can be described as primitive plants (thallophytes) and lack well-defined structures.. Microalgae exhibit a wide range of reproductive strategies including asexual cell division, vegetative reproduction and sexual reproduction (Chinnasamy *et al*., 2012). They are unicellular micro-organisms that can be categorized as prokaryotes and eukaryotes. According to Marmor and Degtjarenko (2014), corticolous algae can be utilized for bio-monitoring of air pollutants due to its resistance towards toxic gases and growth limiting effects of air pollutants (Tastan *et al*., 2012). Therefore, terrestrial microalgae is very useful in monitoring the effect of pollutants on aquatic life forms (Torres *et al*., 2008).

Air pollution is a condition where air pollutants such as Carbon dioxide can be found at high concentration which is above the normal range in the environment. The major pollutants monitored in Malaysia are Ozone (O_3), Nitrogen dioxide (NO_2), Carbon monoxide (CO), Sulphur dioxide (SO_2) and particulate matter (PM10) (Azmi *et al*., 2010). Air quality monitoring is part of the strategy to prevent air pollution in Malaysia. The reports of the air quality monitoring in several large cities in Malaysia cover

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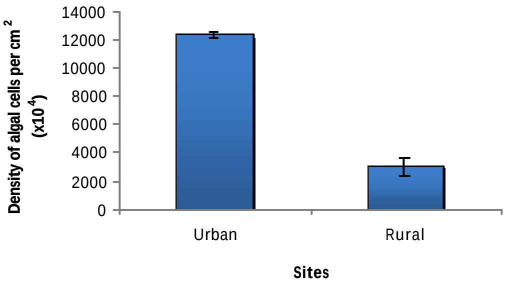

Fig. 1: Emission of atmospheric pollutants in Malaysia in the year 2012, shown in percentage (Department of statstics Malaysia, 2013)

air pollutants such as Carbon monoxide (CO), Sulphur dioxide (SO_2), Nitrogen dioxide (NO_2), Ozone (O_3) and suspended particulate matter (SPM). The sources of air pollution are typically from motor vehicles, industrial emissions and open burning sources. Compared to other sources, motor vehicles are the main cause of air pollution (Afroz, 2003). According to Department of Statistics of Malaysia (2013), the major air pollutants found in the atmosphere is carbon monoxide (CO) and nitrogen dioxide (NO_2).Figure 1 shows the emission of pollutants to the atmosphere in percentage.

Epiphytic terrestrial algae acquire nutrients directly from substances dissolved in surrounding moisture through diffusion. It also capable of absorbing and retaining pollutants in larger amount compared to other plant groups. Similar to epiphytic lichens, algae live primarily on atmospheric moisture and nutrients. Therefore, they are very responsive towards the changes in air quality (Nash, 2008). Under acid pollution, the pH of the tree bark decreases whereas, dust pollution leads to the increase in pH of the tree bark. Extremely high or extremely low pH is toxic to these lower plants. As pH of tree barks change due to pollution, the epiphytic flora are also affected Otnyukova and Sekretenko, 2008). This current study focuses on the effect of carbon monoxide (CO) on the density of microalgae. The objectives of the study are as follows:

- To compare the species richness of the epiphytic terrestrial microalgae from the urban and the rural areas
- To determine the relationship between carbon monoxide and epiphytic terrestrial microalgae

MATERIALS AND METHODS

Sampling stations: The sampling site was conducted at four study sites located at Kuala Selangor, Rawang and Pelabuhan Kelang in the state of Selangor and Port Dickson in Negeri Sembilan. Kuala Selangor and Port Dickson representing the rural area whereas Pelabuhan Kelang and Rawang representing the urban area. These category was created based on the Air Pollution Index of each sites.

The sampling station in Kuala Selangor is located at Kuala Selangor Nature Park while the sampling station in Port Dickson is located at Tanjung Tuan Recreational Forest. Both sampling sites are thick dense forest with minimum exposure to atmospheric pollutants. The urban sites chosen for this study particularly of Pelabuhan Kelang is located at Pendamaran Industrial Estate where this area is well-known for its wide expanse of factories and industrial plants which emits an array of atmospheric pollutants. Similarly, Rawang is also known to accommodate large number of motor vehicles since the sampling station was located at the Rawang Rest and Relax area, on the major highway of North South Highway with over 200,000 cars a year (Malaysian Ministry of Transport, MOT, 2012). Figure 2 shows the map of the study sites.

Quadrat sampling method : Two quadrats of 10 cm x 10 cm were laid on the bark of tree. Five trees were selected at each sampling site making a total of 40 samples of epiphytic terrestrial algae. Samples were collected by brushing off the algae within the quadrat using wet cotton wool. The cotton wool with algal colonies was put in a 100ml specimen bottle containing 30ml distilled water.

Algal Count: The density of microalgae at each site was quantified using a hemocytometer. 10μL of microalgae sample was transferred into the hemocytometer chamber by touchingthe cover slip on the hemocytometer at its edgewith the pipette tip and capillary action will allow the chamber to be filled. The microalgae cells were counted only on the numbered quadrat. Three replicates of count were made for each sample. The density of cells obtained was then converted to cells per cm^2. The counting follow the following formula :

Cells density (in cells/ml) = average count x 10,000 x dilution factor of original cells

Species identification: 10μl of the diluted microalgal samples was placed on a slide and covered with a cover slip. The slide was examined under the microscope. The species were determined by observing the morphology of the microalgae seen under the microscope and compared to the images from The Freshwater Algal Flora of the British Isles and world's algal listings database (www.algaebase.org) as references for identification.

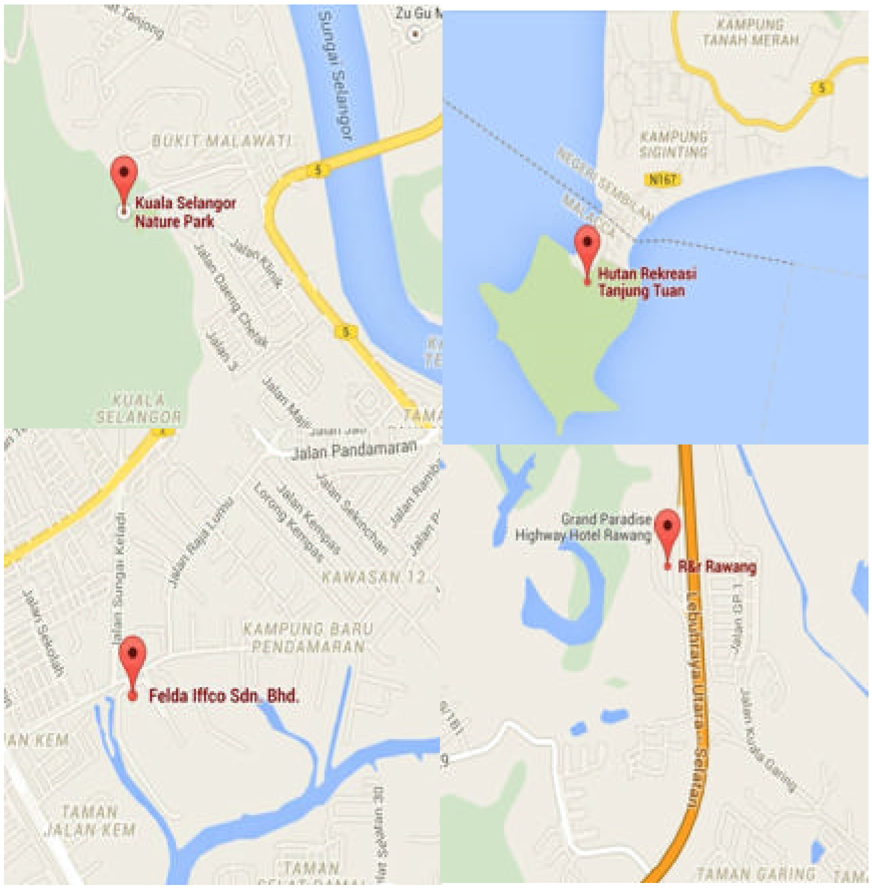

Fig. 2: Location of sampling sites consisting of 2 urban sites and 2 rural sites. Rural sites: a) Kuala Selangor Nature Park, b) Tanjung Tuan Recreational Forest, Urban sites, c) Klang Industrial Estate, d) Rawang on the North South Highway (Adapted from Google Map, 2015)

Concentration of Carbon Monoxide in Sampling Sites: The value of concentration of atmospheric pollutant was obtained from Department of Environment of Malaysia, Ministry of Natural Resources and Environment. The data obtained was for Kuala Selangor, Port Dickson, Rawang and Pelabuhan Kelang from the year 2000-2013.

RESULTS AND DISCUSSION

Species Richness of Epiphytic Algae in all Sampling Sites: Results showed that four different algal species was found in the urban sites compared to the rural ones with three algal species (Table 1 and Fig. 3). Jaccard Similarity Index showed 40% similarity between the rural and urban sites. The low percentage depicts that species composition in the urban and rural sites is similar by only 40%.

Interestingly, the green algae, Trentepohlia umbrina was found at all sites regardless of the level of pollution.

Table 1: Four different algal species

Sites/Location of sites	Algal species	Density of cells± SEM $(\times 10^4)$
Rural		
Kuala Selangor Nature Park	*Desmococcus olivaceus*	2310 ± 26.71
	Klebsormidium sp.	120 ± 2.23
	Trentepohlia umbrina	30 ± 0.20
Tanjung Tuan Recreational Forest	*Desmococcus olivaceus**	3660 ± 34.12
	Trentepohlia umbrina	30 ± 0.40
Urban		
Pendamaran Industrial Estate	*Trebouxia arboricola*	12120 ± 32.99
	Trentepohlia umbrina	30 ± 0.20
Rawang	*Desmococcus olivaceus***	12390 ± 60.06
	Trentepohlia umbrina	30 ± 0.75
	Chlorarachniaceae (identified to family level only)	150 ± 3.27

This green algae was known to be cosmopolitan with wide tolerance range. The density of this species however was on the low side with only an average of 30±0.20×104 cells/cm^2. The most abundant species for both the rural and the urban sites is Desmococcus olivaceus. The density of this algal species was significantly higher in

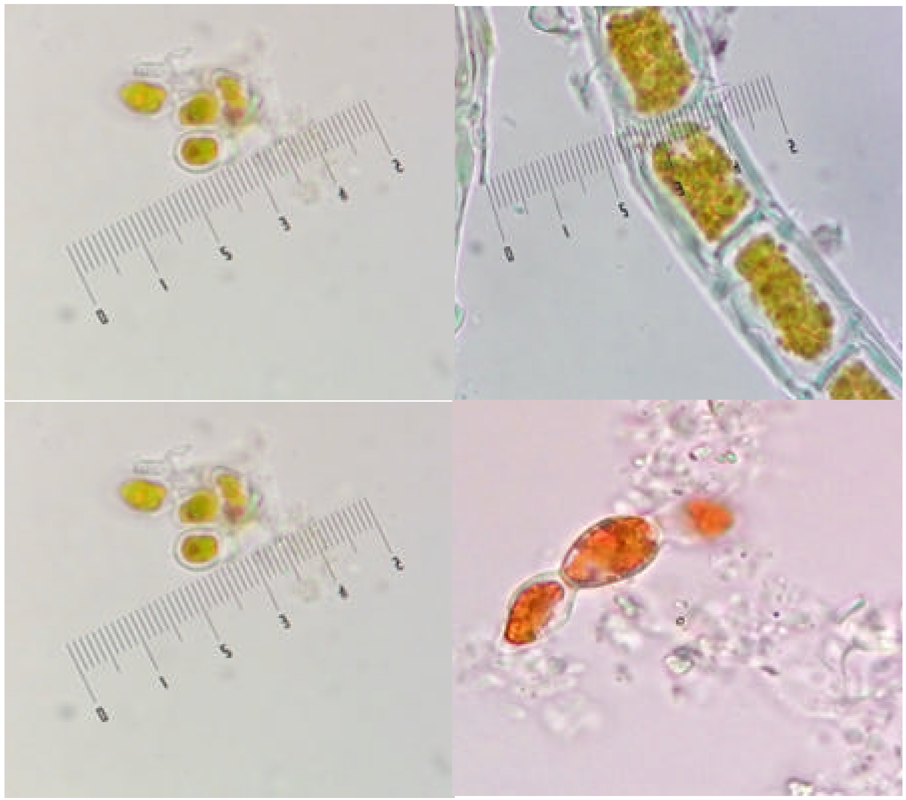

Fig. 3: Epiphytic terrestrial algae inhabiting all sampling sites. Images was observed under digital light microscope at 100x magnification. 1) Trebouxia arboricola, 2) Klebsormidium sp., 3) Desmococcus olivaceus, 4) Trentepohlia umbrina

the urban sites with Rawang recorded up to 12390±60.06×10^4 cells/cm^2, 3-fold higher than the highest density in the rural site at only 3660±34.12×10^4 cells/cm^2, in Tanjung Tuan Recreational Forest. Besides *D. olivaceus*, another algal species that proliferate in the polluted environment is Trebouxia arboricola with algal density of 12120±32.99×10^4 cells/cm^2. This data showed that the urban sites with higher concentration of pollutants probably provide nutrients in terms of atmospheric gases for epiphytic terrestrial algae.

Microalgal Density in Urban Area and Rural Area: Results shows that there is significantly higher density of algal cells/cm^2 at the urban sites compared to the rural sites ($p<0.005$) (Fig. 3). This is probably due to the ability of the microalgae to tolerate to pollutant by enhancing the growth of the microalgae by supplying nutrients to the algal cells (Novakova and Neustupa, 2015).

As for the rural area which is Kuala Selangor and Port Dickson, there are lower levels of pollutants compared to Pelabuhan Kelang and Rawang (urban). Therefore, the algal density in the rural sites are significantly lower than the urban sites.

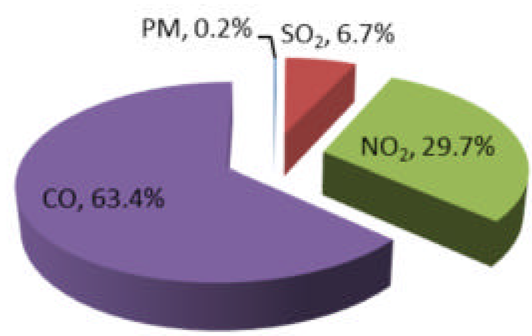

Fig. 4: Mean algal density in the urban and rural site

Relationship between concentration of carbon monoxide and algal density: Table 2 below shows the density of algal cells and concentration of carbon monoxide (CO) in each respective sites. Higher concentration of carbon monoxide was recorded in both polluted sites (urban) namely Pelabuhan Kelang and Rawang at 11.815±0.50 and

8.290±0.21 μg m^{-3} respectively. Concurrently, the increase in CO concentration triggered the increase in algal density where both sites recorded mean algal density of 12150±33.20 and 12150±33.20 cells/cm^2.

The rural area which recorded much lower CO concentration 4.854±0.19 μg m^{-3} in Tanjung Tuan Forest Reserve and only 0.500±0.01 μg m^{-3} in Kuala Selangor Nature Park showed that both rural sites positively showed lower CO concentration than the urban sites. True to our hypothesis, lower CO concentration support lower algal density when this sites recorded mean algal density of <5000 cells/cm^2 compared to almost 13000 cells/cm^2 for the urban sites.

Pearson Correlation statistical test showed that there is strong positive correlation (correlation coefficient of 0.901) between density of algal cells and concentration of carbon monoxide (Fig. 4).

This further support the earlier statement which stated that higher concentration of CO will give rise to higher algal density. General knowledge that carbon dioxide (CO_2) is an essential part for the growth of microalgae and is utilized for the process of photosynthesis (Stepan *et al*., 2002), help to understand why similar function is being observed when CO concentration is on the increase. Principally, CO and CO_2 are positively correlated. Therefore, when higher level of CO_2 promotes the growth of microalgae, it explains the higher density of algal density when CO increased. This result is in agreement with previous study conducted by Wang *et al.* (2010).

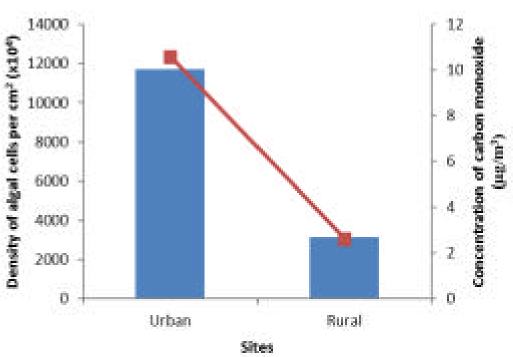

Fig. 4: Mean algal density in the urban and rural area with concentration of carbon monoxide for each area respectively. The bar chart represents the density of algal cells/cm2 while the line graph represents the concentration of carbon monoxide in μg/m3

CONCLUSION

In conclusion, there are a total of five different species of microalgae recorded from the study. Urban sites recorded higher species richness compared to the rural sites. Desmococcus olivaceus is regarded as the most tolerant since it was abundant at the urban sites and also dominant in the rural sites. Carbon monoxide is found to be positively correlated with algal density with correlation coefficient of 0.957.

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