Air Pollution and Shading as Possible Factor Affecting Number of Algal Cells (Chlorophyta: *Coccomyxa confluens*)

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Abstract: Terrestrial algae, one of the many important living things for the earth's ecosystem are abundant in large spectrum such as on tree barks, stones, walls and plastics. The objectives of this research were to assess the effect of shading towards the number of algal cells inhabiting 30 free standing trees. Besides that this study also looks into the number of algal cells in polluted and unpolluted environment. The results of this study showed that epiphytic terrestrial algae were found to grow abundantly in polluted environment as opposed to unpolluted ones. The algae are believed to be able to tolerate high amount of air pollutants such as carbon dioxide, carbon monoxide and many nitrogenous gases. Shading also appeared to influence the number of algal cells where the area with less shade provide better living ground for the algae. The number of algae is directly proportional to the amount of light they received. Therefore, this study provides a useful baseline data on some pollutant-tolerant algal species.

Key words: Abundance, algae, banting, Kuala Lumpur, non-polluted area, polluted area

INTRODUCTION

Algae are a non-vascular plants or also known as lower plant which sustained without a vascular system namely the xylem and phloem. They possess simpler tissues specialize for internal transport of water. Lower plants consist of some of the oldest organisms on earth. They play an important role in ecosystems as primary producers and as nutrient and water recyclers. These plants are divided into two distantly related groups; bryophytes and algae. There are about 25,000 different species of bryophytes in the world today, include mosses, liverworts and hornworts. Although, these plants are small in size, they are one of the largest groups of land plants and can be found almost everywhere in the world (Akiyama, 2010). Bryophytes are regarded as transitional between aquatic plants like algae and higher plants like trees. They are extremely dependent upon water for their survival and reproduction. Some bryophytes, however are able to survive in areas with little or no rainfall (Akiyama, 2010). However, this study is focusing on another group of lower plant, the algae.

Algae are one of the most robust organisms on earth and are able to grow in a wide range conditions. They are usually found in damp places or bodies of water. They are also common in terrestrial as well as aquatic environments. However, terrestrial algae are usually rather inconspicuous and far more common in moist, tropical regions than dry ones because algae lack of vascular tissues and other adaptations to live on land. Mostly common algae that can be found in essentially every type of terrestrial environment are cyanobacteria and eukaryotic algae (Broady, 1996; Barkman, 1958). They are so called terrestrial algae in the sense that they are not dependent on liquid water (Rindi *et al.*, 2009). They possess a number of genes that code for specific substances that maintain cellular integrity, structure and viability through extreme transitions. Terrestrial algae play important roles in every ecosystem. They contribute to the fertility and stability of soils everywhere, through fixation of carbon andnitrogen, release of organic compounds and binding together with oil particles to reduce soil erosion.

The objective of the study is to compare the growth of algae in both polluted and less-polluted area. The area chosen is KLCC (Kuala Lumpur City centre) for polluted area and Banting representing the less-polluted area. Kuala Lumpur City centre is a multipurpose development area in Kuala Lumpur (Afroz *et al.*, 2010). The area is located around Jalan Ampang, Jalan P. Ramlee, Jalan Binjai, Jalan KiaPeng and Jalan Pinang. KLCC comprise 100 acre land area with mixed developments in various stages of construction. The area is divided into several plots of land, each with specific purpose and is congested

with public transportation, mainly the monorails and taxis. Besides that it is also a bus hub for Rapid KL bus network, the largest public transport operator in Kula Lumpur. Other factors that detract the air quality in Kuala Lumpur are forest fires, vehicle emissions and industrial pollution. Thus, the air pollution level has become a problematic issue in Kuala Lumpur. Based on official Air Pollutant Index (API) gathered from Department of Environment, Ministry of Natural Resources and Environment, Malaysia, the API of Kuala Lumpur shows an unhealthy index which is at 142c. On the other hand, Banting is a small town in the district of Kuala Langat, Selangor, about 70 km from Kuala Lumpur. It is located on the banks of Langat river and consists of beaches, hills, forest and farms. The API of Banting is considered as good much lower than KLCC with only 42c.

Literature review

Air pollution: Air pollution is created from incomplete carbon reactions, unburned hydrocarbons or other elements present in the fuel or air during combustion. These processes produce pollutants of various species (Afroz *et al.*, 2010). The main pollutant is in a form of gases. Recently, air pollution had become a major problem in the world. This is mainly due to the number of motor vehicles that had increased from 0.3 million in 1951, 37.2 million in 1997 (DOE, 2007). In Malaysia, Afroz *et al.* (2010) reported that the three major sources of air pollution are mobile sources (70-75% of total air pollution), stationary sources (20-25%) and open burning sources (20-25%). The major air pollutant is carbon monoxide and nitrogen dioxide (DOE, 2012).

Air Pollutant Index (API) is a system used in Malaysia to monitor level of air pollutant in the air. This system normally includes major air pollutant such as Ozone (O3), Carbon monoxide (CO), Nitrogen dioxide (NO2), Sulphur dioxide (SO2) and suspended particulate matter of <10 microns in size (PM10). The ranges of indexes were categorised as good, moderate, unhealthy, very unhealthy and hazardous (DOE, 2013). Good API was ranged from 0-50c, moderate was from 51-100c, unhealthy was from 101-200c, very unhealthy was from 201-300c while hazardous API was >300c.

Taxonomy and morphology of terrestrial algae: Terrestrial algae have a very simple and uniform habit such as single cells, sarcinoid and uniseriate filaments. For single cells, the examples were *Chlorella* sp., *Chlococcum* sp., *Stichococcus* sp. and *Trebouxia* sp. For sarcinoid habit, the example were *Coccomyxa* sp., *Apatococcus* sp., *Desmococcus* sp., *Chlorosarcina* sp. and

Chlorokybus sp. They were in packet-like colonies formed by a limited number of cells. For uniseriate filaments, they could be in branched and unbranched forms. The species such as *Klebsormidium* sp., *Printzina* sp. and *Trentepohlia* sp., exhibits this kind of habit (Akiyama, 2010). All this habits offers characters useful for taxonomic and systematic purposes (Rindi *et al.*, 2009). Taxonomiccriteria at the species level are mainly based on shape and size (length and width) of vegetative cells, presence of hair-like cells (setae), branching pattern, position and morphology of reproductive structures. Based on Thompson and Wujek (1997), some of the features used for taxonomic purpose are unstable and can vary in relation to ecological conditions.

Habitat: Algae are usually mainly known from marine and freshwater habitats. But, they also occur in a wide variety of terrestrial environments (Hoffmann, 1989). About 800 species of algae are known to occur in terrestrial environments. They form conspicuous growths in several surface types including rocks, urban walls, metals, tree barks, leaves and animal hairs (Lopez-Bautista *et al.*, 2007). Algal species living on tree bark are known as aero-terrestrial algae. Besides that they also occur in the most extreme habitats such as walls of urban buildings (Rindi *et al.*, 2010), biotic crusts in hot deserts (Lewis and McCourt, 2007) Antartic snow (Broady, 1996) and air at 2,000 m height (Sharma *et al.*, 2007).

Abiotic factors that affects the growth of algae: A study by Aresta (2003) stated that light, humidity, temperature and suitable nutrients such as carbon, nitrogen and phosphorus are the factors that effect the growth of algae. Another study by Fogg (1975) stated that algae require a supply of inorganic nutrients, sufficient light and favorable temperature to grow. Light and humidity are considered the most influential abiotic factors on the growth and development of terrestrial algae (Islam, 1960). The diversity of different algal groups is mainly influenced by light conditions (Neustupa and Skaloud, 2008). Kitaya *et al.* (2005) investigated the effects of humidity and light intensity on cellular multiplication of microalgae. The results demonstrated that the highest multiplication rate of the algae was at light flux of about 100 μmol m^{-2} sec^{-1} and air humidity between 40-50%. Sufficient amount of inorganic nutrients is also important. The growth of many species of algae is limited by the availability of inorganic nutrients such as nitrogen or phosphate (Lapointe, 1989; Larned, 1998). A study by Bremer (1985) states that algae can take up dissolved NOx as their source of nitrogen which aid in their growth.

Shading: Light conditions directly affect the growing and photosynthesis of algae. They need a light for productive photosynthesis. Photosynthesis is photochemical phase which produce ATP and NADPH. However, they also need dark condition for biochemical phase in synthesizing essential molecules for growth (Maryam *et al.*, 2012). Experimental investigations by Khoeyi *et al.* (2011) reveal that the increase in light duration and light intensity is directly proportionate to increase in number of algal cells. Insufficient light may lead to growth limiting or photo-oxidation and inhibition.

Another studies reported that sulphur dioxide can induce visible injury to leaves. This will then leads to reduction in photosynthetic pigments. Lewis and McCourt (2007) stated that sulphur dioxide can inhibit metabolic processes of plants. Air pollutants give direct impact on their primary metabolic functions. The most sensitive species may become locally extinct in urban areas or near industrial facilities. This area contains a high level of air pollutants in the air. However, some pollution tolerant species will survive. They may even flourish in sites with poor air quality (Havens, 2007). If air is very badly polluted, there may be no lichens present, just green algae may be found. If the air is clean, hairy and leafy lichens will become abundant (Havens, 2007).

MATERIALS AND METHODS

Study sites: Our sampling sites are located in Kuala Lumpur City Centre (KLCC) and Banting, Selangor (Fig. 1 and 2). The study has been conducted in two different areas which are the polluted area, represented by KLCC and the non-polluted area, represented by Banting. These two areas are 70 km apart. According to the Malaysia Department of Environment (DOE), KLCC recorded the Air Pollutant Index (API) of 142c with the temperature of 35°C and humidity of 44% meanwhile Banting recorded an API of 42c with the temperature of 36°C and humidity of 57%.

Samples collection, algal density and species identification: Algal samples were collected at two different aspects in each sampling site; the East and the West. Samples from the east are representing the algae inhabiting the non-shaded area while samples from the west are representing algae in shaded area. The direction was determined using a compass which was set up according to the position of the sun. The samples were collected using cotton bud dipped in the distilled water. Then, the samples were placed into 20 mL sampling bottles contained of 10 mL distilled water. All samples were preserved in the refrigerator at 4°C. The method used for estimating the abundance of algae was using the quadrat sampling method. A 15×15 cm quadrat was placed on each tree at 1.5 m from the ground. The algae in the quadrat were scraped out and quantified using a haemacytometer. This method allows estimation of absolute density (number of individuals per unit area within the study site). Species of algae was observed under light microscope and the morphological characters were noted to aid in species identification. The species were identified using a taxonomic book "The Freshwater Algal Floral of the British Isle" and also referring to the established algal database "www.algaebase.org".
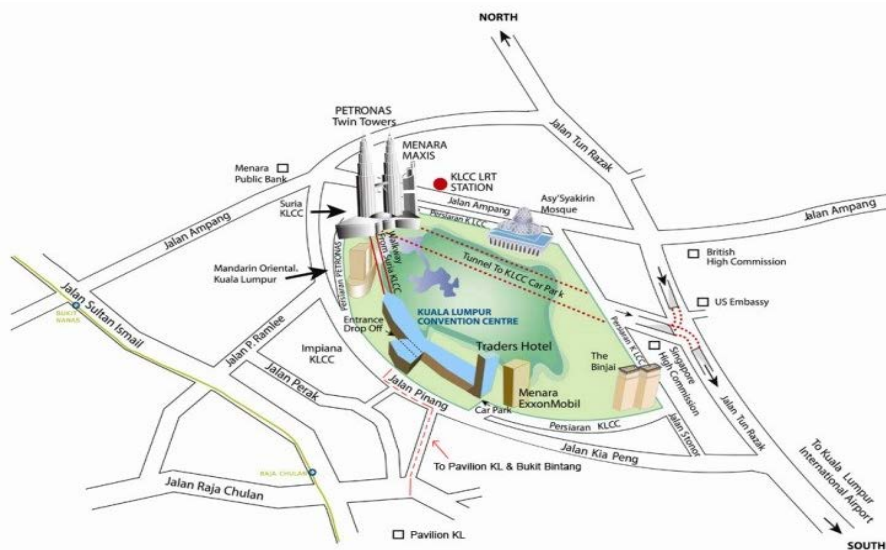

Fig. 1: Sampling site located in KLCC which represented as polluted area

Fig. 2: The sampling site located in Banting, Selangor, represented as non-polluted area

RESULTS AND DISCUSSION

Density of algae in polluted and non-polluted area: Result showed that the algal density in polluted environment was notably higher compared to the non-polluted environment (Fig. 3). Statistical analysis also showed that there was significant difference of number of algal cell in polluted and non-polluted environment ($p<0.05$). We hypothesized that the epiphytic terrestrial algae requiring and utilizing the supply of carbon that was readily available in polluted environment to produce energy. Previous study showed that limited number of carbon will affect algal growth. On some extreme cases, the absence of carbon lead to no growth as insufficient supply of carbon is regarded as a limiting factor in algal productivity (Brown, 1996). Apart from carbon, algae also require the supply of nitrogen (Goldman and Horne, 1983). Nitrogen helps in the metabolic processes of the algae. Similar to carbon, insufficient level of nitrogen could also limit the number of algal cells. Higher amount of carbon and nitrogen in the polluted environment are among the factor that help in the growth of epiphytic terrestrial algae compared to non-polluted environment which contain relatively lower level of carbon and nitrogen (Aresta, 2003; Cardon *et al.*, 2008). It is safe to say that the algae in polluted environment have better ability to tolerate the air pollutant.

Density of algae in shaded and non-shaded environment in each site: Figure 4a and b showed the number of algal cells in shaded and non-shaded environment collected in
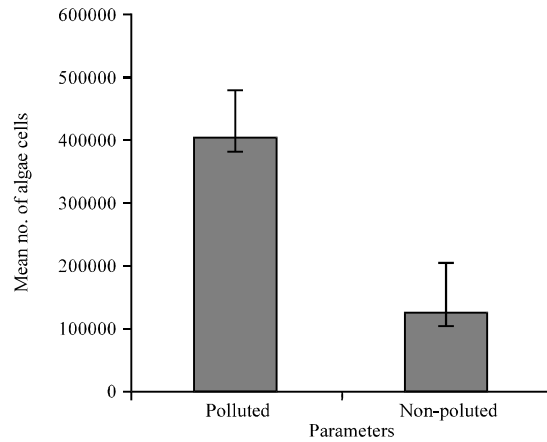

Fig. 3: Number of algal cells in the polluted and non-polluted environment. Data are expressed in cells/mL

polluted environment and in unpolluted environment, respectively. Results showed that the number of algal cells was significantly higher in non-shaded area compared to shaded area for both polluted and non-polluted environments. The result ultimately leading to a statement that both polluted and unpolluted environments are supporting the evidence that epiphytic terrestrial algae algae thrive better in non-shaded environment.

The polluted environment showed a contrasting number of algae between shaded and non-shaded area in the said environment. Thus, the data was significantly different ($p<0.05$). However in unpolluted environment, the number of algae in shaded and non-shaded area was

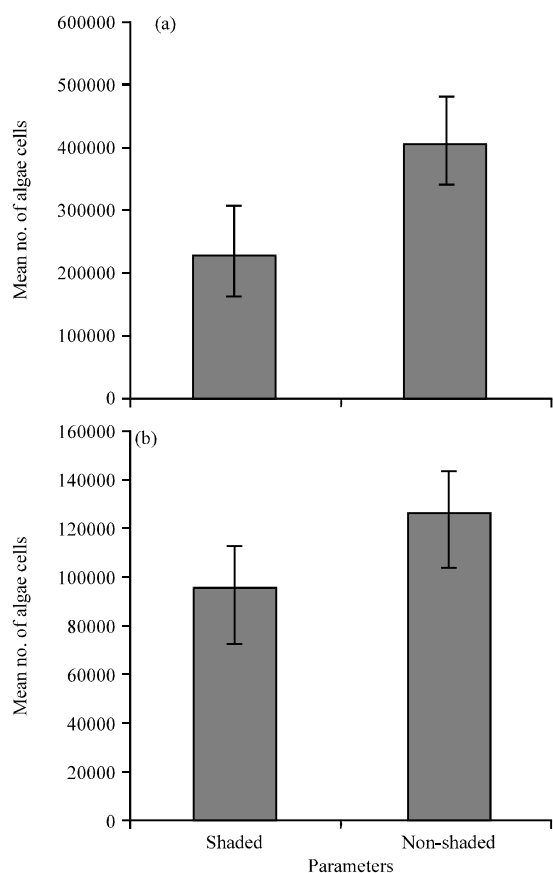Fig. 5: *Coccomyxa confluens*

Fig. 6: *Trentepohlia aurea*

Fig. 4: Number of algal cells (data are expressed in cells/mL) in shaded and non-shaded environment in: a) polluted area b) unpolluted area

not statistically different. Non-shaded area received higher amount of light at higher intensity compared to shaded area. Most plants including algae required light as their source of energy to produce their own food (Maryam *et al.*, 2012; Havens, 2007). Hence, the algae that received sufficient light will have rapid growth compared to the algae that did not received enough light (Khoeyi *et al.*, 2011; Kitaya *et al.*, 2005).

Algal species identification: There were two species of algae observed in this study. They were *Coccomyxa confluens* (Fig. 5) and *Trentepohlia aurea* (Fig. 6). In both polluted and unpolluted environment, the most dominant algae were *Coccomyxa confluens*. This species were found growing abundantly on the bark of trees. *C. confluence* are known to be able to tolerate pollutant well, especially that of carbon dioxide and nitrogen oxide. Besides that this study also recorded another algal species that belongs to Trentepohliales called *Trentepohlia aurea* (Hoffmann, 1989). This species

can also be found in polluted environment. However, the number was relatively small. Even though, *T. aurea* are known to be able to live in polluted environment, this species are less viable compared to *C. confluens* which is more robust.

CONCLUSION

As a conclusion, the green algae *Cocomyxa confluence* can be regarded as pollutant tolerant species because this species grow abundantly in polluted environment. Inorganic nutrients such as carbon and nitrogen in the air pollutants are believed to aid their growth. However, the amount of light received by the algae is also a primary factor that contributes to their growth. The number of algae was found to directly proportional to the amount of light they received. The number of algae is higher at non-shaded area compared to shaded area regardless of their environment.

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