Academic Fora

FULL PAPER PROCEEDING
Multidisciplinary Studies
Available online at www.research-cluster.com

Full Paper Proceeding ECBA-2017, Vol. 03 – Issue 10, 1-6

ISBN 978-969-683-507-3

ECBA-17

Acid Rain Simulation and its Effect on the Density and Species Composition of Epiphytic Terrestrial Algae

Asmida Ismail[1*], Afifah A.Kamal Bahrin[2], Amyrul Rafiq Zulkifli[3], Mohd Nizam Mohd Said[4], Ahmad Ismail[5], Sarah Diyana Marzuki[6], Ihsan Alwi[7], NorDiana Bakti Mohd Yusoff[8]

[1,2,3,6,7,8] Universiti Teknologi Mara, Malaysia, [4,5] Universiti Kebangsaan Malaysia, Bangi,Malaysia

Abstract

Acid rain is liquid precipitations with pH lower than 5.6 which contain harmful elements such sulfur dioxide, nitrogen oxides, nitric and carbon dioxides. The increasing trend of acid precipitation imposed a negative impact on ecosystem, including lower plants such as algae. In this study, acid precipitation was simulated in accordance to natural acid rain comprises of sulfuric acid, nitric acid, and acetic acid which represents sulfur dioxide, nitrates, and acetic acid respectively. Distilled water act as control. A total of 52 quadrats were placed on 20 trees where 12 quadrats were taken to determine the initial algal density and species composition before the treatments were taken place. Two of the quadrats act as negative control where measurement is taken without any treatment applied. Each respective treatment was sprayed to 10 quadrats, once a week for 8 weeks. After 8 weeks of simulation, sulfuric acid showed the most significant decrease in algal density, lethal to algae which resulted in more than 99% reduction from 110.3 x 106 ± 10.17 cells/cm2 to almost zero density. Meanwhile acetic acid showed positive increment by 5% from 76.0 x 106 ± 13.05 cells/cm2 to 79.4 x 106 ± 12.92 cells/cm2. Declining from 97.7x 106 ± 11.78 cells/cm2 to 23.9 x 106 ± 5.0 cells/cm2, nitric acid shows 75% decrement in the algal density. As expected, the control quadrat of this experiment showed an increment of 4% from 116.3 x 106 ± 8.82 cells/cm2 to 120.1 x 106 ±10.75 cells/cm2. Correspondingly, this study also found that Desmococcus sp. is the dominant species apart from Stigeoclonium sp. and Ulothrix sp. To conclude, sulphur dioxide and nitrogen dioxide caused a lethal action with total reduction and 75% reduction algal reduction respectively while carbon dioxide only minimally increased algal density to less than 10%. It is recommended to substitute nitric acid with ammonium nitrate to represent nitrogen dioxide in precipitation.

Keywords— Acid Rain, Terrestrial Algae, Sulfur Dioxide, Acid Deposition, Species Composition

Introduction

Acid deposition is caused by the emission of primarily three atmospheric gasses namely, SO2 (sulphur dioxide), NOx (nitrogen oxides) and CO2 (carbon dioxide) and known to have pH lower than 5.6 (Behera, Mallick, Rautray, Tiwari, & Mishra, 2014). It had become a detrimental phenomenon with the advent of industrialization and recognized as international problem.

Specifically, Asia is experiencing a rapid growth of atmospheric air pollutants (Kato & Akimoto, 1992; Rodhe, Galloway, & Dianwu, 1992) with an increase in sulfur dioxide issuance corresponding the domain's extending energy needs (Foell et al., 1995; Ling, Jiming, & Mingming, 1995). According to Huo, et al. (2012), the degree of pH of rain water is determined by the particles in the atmosphere and the rainfall intensity. It is known that pollutant may act directly on the organisms or indirectly through alteration of the physical environment, for instance in case of acid rain, organisms may reduce the pH of a water body, in order to survive acidity (Bourdeau & Treshow, 1978).

Algae are suitable for assessing the degree of pollution and air quality of the atmosphere because the generation times are much quicker than lichens, bryophytes, or vascular plants. Therefore, terrestrial algae can act as sign of environmental changes significantly faster than other taxa (Marmor and Degtjarenko, 2014).Thus, any effect on the lower level of the food chain will also have consequence on the higher level (Joubert, 1980). In environmental study, epiphytes are the most excellent candidates for such a project due to their high sensitivity to atmospheric pollutants (Lewis, 2012). By analyzing the growth of algae with certain important parameters, it could indicate whether the area is high in pollutants and vice versa. Species that is tolerant and sensitive to acid deposition could be identified. This project is a pilot study which demonstrates variability response of algae with different types of atmospheric pollutant precipitation. The preliminary data will initiate further research on epiphytic algae and their response to atmospheric pollutant. Thus, the objective of this study is to determine species composition of terrestrial algae prior to acid rain simulation and to identify species of algae tolerant to acidity. In addition to that, this study also focused on assessing the dynamic of algal density before and after simulation of acid precipitation.

*All correspondence related to this article should be directed to Universiti Teknologi Mara, Malaysia
Email: asmida@salam.uitm.edu.my

Materials and Methods

Selection of Sites and Samples

The experiment was carried out at Taman Paku Pakis, National University of Malaysia, (UKM) with the coordinates of 2°91’ 97.0 N 101° 78.14° E. This recreational and education park comprises of 8 hectares. The coordinate for Treatment 1 (T1) is 2°55’24.36 N 101°46’58.37° E. Coordinates 2°55’23.75 N 101°46’ 58.62° E for Treatment 2 (T2), 2°55’23.58 N 101°46’59.20° E for Treatment 3 (T3) and 2° 55’24.42 N 101°46’58.82° E coordinates for Treatment 4 (T4). A total of 20 established trees with similar Diameter at the Breast Height (DBH) were selected.

Preparation of Acid Rain Simulation

Simulation of acid rain to represent atmospheric pollutants in liquid form (precipitation) was formulated by adjusting the treatment to the acquired pH.

i. Acetic acid to represent CO_2 in precipitation: 1000 mmol of acetic acid, poured into 200 ml distilled water and adjusted to pH 5.6.
ii. Nitrates to represent nitrogen dioxide: 1000 mmol of concentrated HNO_3, added to 200 ml distilled water (Čučulović et al., 2014), adjusted to pH 5.1.
iii. Sulphuric acid to represent SO_2: 0.193 mmol of H_2SO_4 added to 200 ml distilled water (Hauck, 2008), pH adjusted to 2.8.
iv. Distilled water encompasses as the control of this experiment.

All treatments including the control ones were sprayed to the quadrats of 10 x 10 cm at a volume of 4 ml for 8 weeks on a weekly basis.

Quadrat Sampling Method

The quadrat sampling method was conducted at the study sites following the Nuffield Foundation Website, London (2011). Three quadrats of 10 cm x 10 cm each were placed on each tree. Algae within each quadrat were collected by scraping the bark surface with wet cotton wool and then placed in a sterile specimen tube containing 40ml distilled water. The tube specimens were shaken vigorously to detach the algae from the wool. To prohibit post-sampling growth, samples were stored in a refrigerator at 1-4ºC.

T1 T2 T3 T4

Figure 1: 10 x 10 cm Quadrats on Tree with Colour-Coded Frame to differentiate different Treatments. T1 for Control, T2 Nitric Acid, T3 Carbonic Acid, T4 Sulphuric Acid

Identification and Quantification of Species

Species identification begins with all algal samples being observed under the digital light microscope. The structure of the algae, the characteristics and the anatomy of the samples was observed and referred to the book of algal taxonomy; The Freshwater Algal Flora of The British Isles and the Algabase website for species identification. Algal density was calculated prior to and after the 8-week simulation using the haemocytometer and light microscope. The samples were pipetted into the wells of haemocytometer using micropipette and the sample was observed and counted under the light microscope.

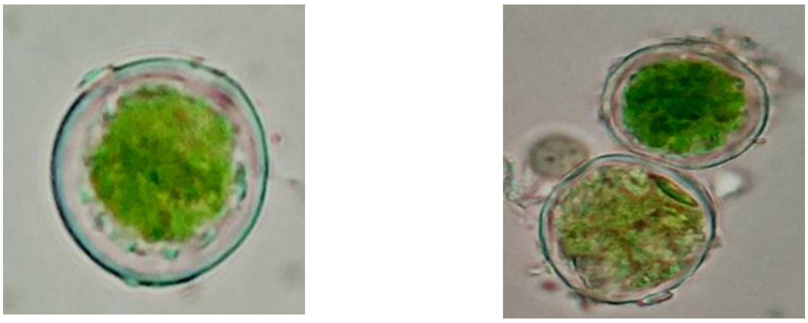
Data Analysis

SPSS software was used to analyze the data obtained. All data are expressed as mean ± SEM (standard error of the mean). T-Test was used as it shows the differences mean within groups and considered statistically significant at $P < 0.05$.

Results

Species Composition of Epiphytic Terrestrial Algae

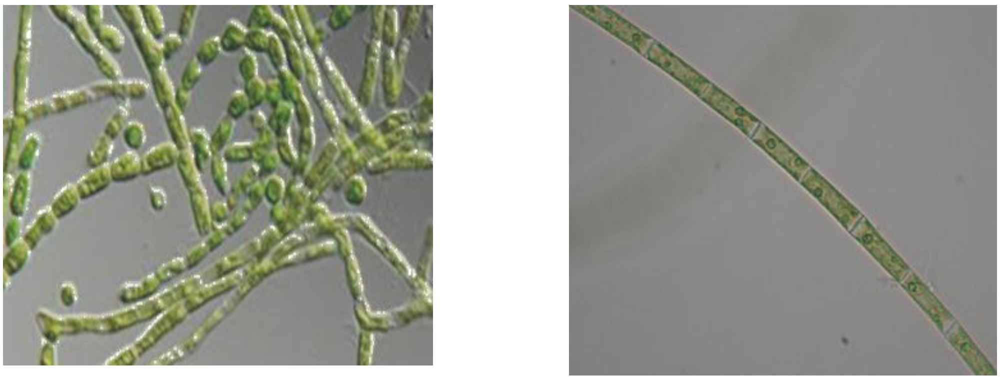
Morphogically, Desmococcus sp. of algae is green, globular in shape, with single or parietal chloroplast. Desmococcus sp. also can be found as single cell and as well as in pair. During observation of the algal density, the Desmococcus sp. was recorded as the dominant species. Note that only dominance species that was being calculated for algal density. However, there is also supplementary species of algae being observed from this study such as Stigeoclonium sp. and Ulothrix sp (figure 3). The number of this supplementary species was relatively small and considered as irrelevant species in this study.



(a) (b)

Figure 2: This Figure shows Desmococcus sp. at 100 × 10 Magnification: (**a**) Individual of *Desmococcus* sp. (**b**) Observed Pair Cells of *Desmococcus* sp.

As for morphological identification of Stigeoclonium sp. is a type of non-motile algae and green in colour. The structure of the Stigeoclonium sp. is filamentous and branched. The cells are normally broad and long. On the other hand, Ulothrix sp. is filamentous green algae. The cells are normally broad and long. The Ulothrix sp. becomes attached to surfaces by a modified holdfast cell. Ulothrix sp. is unicellular type of algae that is characterised by the ring or the band like chloroplasts with one to several pyrenoids. Figure 3 below shows the Stigeoclonium sp. and Ulothrix sp under the microscope.



(a) (b)

Figure 3: This Figure shows Supplementary Species at 100 × 10 Magnification: (a) Stigeoclonium sp. and (b) Ulothrix sp

Algal Density

Species identification found that sulfuric acid gives the most significant effect by diminishing more than 95% of algal density compared to other type of pollutant. Distilled water that act as control gives an increment of 4% followed by acetic acid 5%. Meanwhile nitric acid shows dynamic shift decrease of 75% percent. The number of algal cells for Treatment 1, which is the distilled water, as the control is 116.3 x 106 ± 8.82 cells/cm2. The number of algal cells after the treatments is slightly increase to 120.1 x 106 ±10.75 cells/cm2 after it was exposed for 8 weeks. For Treatment 2, the number of algal cells before the treatment was 76.0 x 106 ± 13.05 cells/cm2 and slightly increased to 79.4 x 106 ± 12.92. In Treatments 3, the number of algal shows huge decline from 97.7x 106 ± 11.78 cells/cm2 to 23.9 x 106 ± 5.0 cells/cm2. From the results, it can be concluded that Treatment 3, caused vital effect to the density of the algae. The percentage of algal density was dropped by 75%. The most remarkable effect is detected in treatment 4, where the initial number of algal cells before the treatment is 110.3 x 106 ± 10.17 cells/cm2. However, after 8 weeks of treatments, it shows that there is no existing number of algal densities at the end of this project.

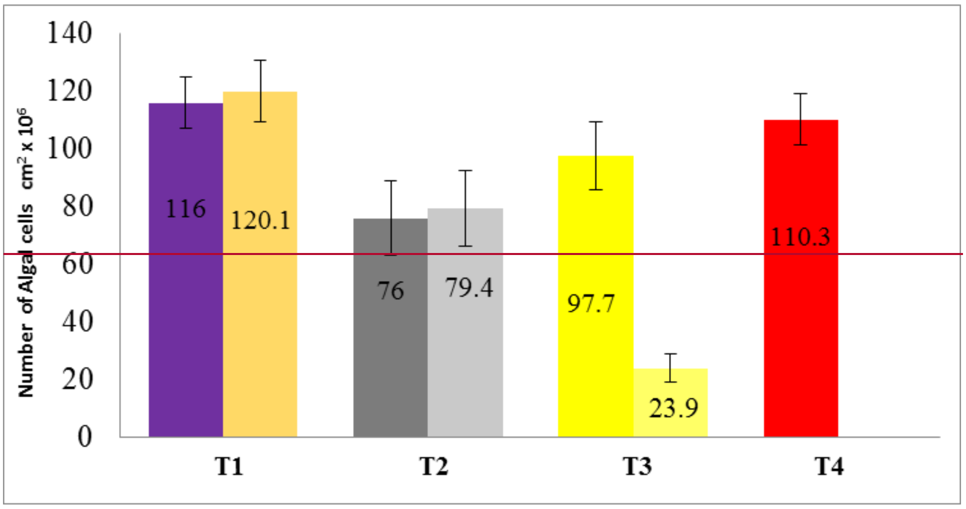


Figure 4: The Dynamic of Epiphytic Terrestrial Algae before and after Acid Rain Simulation

Table 1:
Density of Algal before and after Simulation of Acid Rain

No of algal cells ($cm^2 x 10^6$)	To represent atmospheric gases	Before	After	% Difference
Distilled water (T1)	Water	116.3± 8.82	120.1±10.75	+ 4%
Acetic acid (T2)	Carbon dioxide	76± 13.05	79.4± 12.92	+ 5%
Nitric acid (T3)	Nitrogen dioxide	97.7±11.78	23.9± 5.0	-75%
Sulfuric acid (T4)	Sulphur dioxide	110.310.17	0.00	-100%

Discussion

Sulfuric acid and nitric acid is a strong acid which shows decrease in algal density because polluting gases inhibit stomata movements, photosynthesis, and growth (Zeiger, 2006). From this study, sulfur had the most detrimental effect. Sulfur dioxide comes in contact with the chlorophyll of the cell and the other constituents of the cells are then converted into corrosive sulfuric acid which immediately destroys the tissues in its vicinity (Bourdeau & Treshow, 1978).

In this study, sulfuric acid had the highest acidity of 2.8 and numerous studies have shown that rainfall less than pH 4 is toxic to the photobiont of lichens, reducing photosynthetic rate and pigment concentration, and leading to plasmolysis and death of the algal cells, especially if the concentration of sulphate in the rain is high (Galloway, Likens, Keene, & Miller, 1982). This explains why in this study, algae that simulated with sulfuric acid lost more than 95% density because the pH is 2.8 which intolerable by the species. Smith, Pitcher, and Wigley (2001) found in their study that sulfur dioxide (SO2) is the fundamental cause of acid precipitation, which adversely influence natural systems besides agriculture and building materials. The bulk of acidity in rain comes from the reaction of sulfur dioxide with hydrogen peroxides in the clouds which produces sulfuric acid (Kulp, 1990). None of the dominant and supplementary species of algae survived the sulfuric rain simulation.

As for nitric oxides, that was represented by nitric acid also caused a significant effect to the density of the algae. The percentage of algal density was dropped by 75% as nitric acid is types of a strong acid with higher association of hydrogen ion concentration that govern the damage of the algae. Therefore, the number of algae cells was reduced (Sikora, 2004). Numerous deadly changes in metabolism of plants that is cause by air pollution lead to external symptoms of injury, which appear only at much higher concentrations. For example, when plants are exposed to air containing nitric oxides, lesions on leaves appear at concentration of 5 ml (Zeiger, 2006). Zeiger, 2006 also state that polluting gases inhibit stomata movements, photosynthesis, and growth. Anyhow, note that the pH of nitric acid solution in this experiment is too high, as normal strong acid should range between pH 3 and below. For future reference, simulation nitric acid solution can be at pH 2.4 as microscopic living organism for instance lichen, Peltigera sp was found to be sensitive at that particular pH (Gunther, 1988).

On the other hand, the results also show that the algae are tolerance towards the changes of the carbon dioxide concentration that was representing by the acetic acid. There is an increase of 5% of algal density after simulation of carbonic acid precipitation. Sant et al, 2015 suggests that the process of photosynthetic efficiency is increase 50% for plants grown in carbon dioxide environments. With the increment of algal density, it can also be said that carbonic acid is a promoting agent of algal growth as it provides source of carbon to the epiphytic terrestrial algae.

Nevertheless, acid precipitation does not usually destroy the trees directly. It acquires few years and decades to really damage the site of a forest and plants itself. The acidity are more likely weakening the trees by limiting the nutrients available to them, damaging their leaves or foliar and exposing them to toxic substances. The outcome of acid admissions on higher plants arises in two ways either through foliage or through roots. The symptoms include direct damage to especially roots and foliage, reduced canopy cover, crown dieback and whole tree death (Singh and Agrawal, 2008). It is more likely that the effect of acid rain is more focused and observed on higher plants. But the effect is also observable on lower plants specifically on epiphytic terrestrial algae. This is because algae also undergoes photosynthesis and acid rain may cause low photosynthetic rate and low stomata conductance in plants (Singh and Agrawal, 2008).

Conclusions

As a conclusion, Desmococcus sp. is the dominant species which can tolerate carbon monoxide well as the species remain to increase despite the application of simulated acid rain in contrast with sulfate and nitrate. Sulfuric dioxide is the most influential atmospheric pollutant and cause major degradation in algal density and species composition of epiphytic terrestrial algae. For a purpose of achieving a better result, the short term treatment should be prolonged to long term treatment. In further research, it is recommended that pH of the tree bark should also be measured before and after acid rain simulation.

Acknowledgments

Authors would like express their gratitude to those who helps and made this project a successful one. This work was funded under PRI 100-RMI/PRI 16/6/2 (4/2015), Fundamental Research Grant Scheme RMI/FRGS 5/3 (145/2013), Ministry of Education, Malaysia, Universiti Teknologi MARA, Shah Alam and Yayasan Sime Darby-UKM (ZF-2015-031).

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