BIOINDICATION OF ATMOSPHERIC HEAVY METAL POLLUTION BY USING MOSSES AT MOUNT KINABALU, SABAH, MALAYSIA

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ABSTRACT

BIOINDICATION OF ATMOSPHERIC HEAVY METAL POLLUTION BY USING MOSSES AT MOUNT KINABALU, SABAH, MALAYSIA

This study was carried out to determine the accumulation capacity of selected mosses as active bioindicators for atmospheric heavy metals (Cd, Cr, Cu, Pb, and Zn) on Mount Kinabalu, Sabah. Ten local moss species were selected, namely *Brachythecium plumosum*, *Breutelia arundinifolia*, *Hypnodendron dendroides*, *Leucobryum javense*, *Leucobryum sumatranum*, *Sphagnum cuspidatum*, *Sphagnum sericeum*, *Spiridens reinwardtii*, *Trismegistia calderensis*, and *Trismegistia panduriformis*. The mosses were collected within the Kinabalu Park and acclimatised in a temperature-controlled room (12-17 °C) for about one week. Subsequently, they were transplanted using nylon moss bags at nine monitoring sites for three months and periodically monitored after 30, 60, and 90 days of exposure. The monitoring sites were divided into three different zones, namely roadside, in park, and on mountain. The concentrations of heavy metals in the desiccated apical shoots of mosses were determined by flame atomic absorption spectrophotometry (F-AAS). The chlorophyll contents of the mosses were determined by ultraviolet (UV) spectrophotometer. The results revealed that Pb element was the highest heavy metal pollutant in the study area. The metal concentration in the moss samples significantly increased with the duration of exposure and was higher at the roadside. The level of heavy metal pollutants were arranged as Pb > Zn > Cr > Cd > Cu. In general, the accumulated metals were significantly correlated (at $p < 0.01$) with the nine monitoring sites and four exposure periods. However, it has no significant correlation with the chlorophyll contents in the mosses, which suggests that other condition have a greater influence on the chlorophyll concentration. *Breutelia arundinifolia* and *Trismegistia panduriformis* were the best bioindicators, among the ten selected species, for atmospheric heavy metal pollutants. In conclusion, local mosses have potential as bioindicators of heavy metals in tropical highlands and could be used in long term monitoring measures.

Keywords: Mosses, atmospheric pollution, heavy metals, bioindicators.

ABSTRAK

Kajian ini dijalankan bagi menentukan keupayaan penyerapan logam berat (Cd, Cr, Cu, Pb, dan Zn) di udara dengan menggunakan lumut jati yang terpilih sebagai penunjuk biologi aktif di Gunung Kinabalu, Sabah. Sepuluh spesies lumut jati tempatan telah dipilih iaitu Brachythecium plumosum, Bruetelia arundinifolia, Hypnodendron dendroides, Leucobryum javense, Leucobryum sumatranum, Sphagnum cuspidatum, Sphagnum sericeum, Spiridens reinwardtii, Trismegistia calderensis, dan Trismegistia panduriformis. Sampel lumut dikutip di dalam kawasan Taman Kinabalu dan dikuarantinkan selama satu minggu di bilik suhu terkawal (12-17 °C). Seterusnya, sampel lumut dipindah-tanam di sembilan kawasan pemerhatian selama tiga bulan menggunakan beg lumut yang diperbuat daripada nylon dan diperhatikan secara berkala pada setiap tempoh dedahan ke-30, 60, serta 90 hari. Kawasan pemerhatian terbahagi kepada tiga zon yang berbeza iaitu jalan raya, dalam taman, serta di atas gunung. Kepekatan logam berat di dalam lumut dianalisis mengunakan alat spektrometer cahaya serapan atom (F-AAS) manakala kandungan klorofil di dalam lumut pula diukur menggunakan alat spektrometer cahaya ultraungu (UV). Hasil kajian mendapati elemen Pb adalah logam berat tertinggi di kawasan kajian. Kepekatan logam di dalam lumut meningkat secara signifikan terhadap tempoh dedahan dan paling tinggi di zon jalan raya. Paras logam berat pencemar boleh disusun sebagai Pb > Zn > Cr > Cd > Cu. Secara keseluruhannya, logam berat yang terkumpul berkolerasi secara signifikan (pada $p<0.01$) terhadap sembilan kawasan pemerhatian dan empat tempoh dedahan. Namun demikian, tiada hubungan kolerasi didapati antara kandungan logam berat yang diukur dengan kandungan klorofil di dalam lumut yang dikaji. Ini menunjukkan terdapat faktor lain yang mempengaruhi kepekatan klorofil. Antara 10 lumut yang dikaji, Bruetelia arundinifolia dan Trismegistia panduriformis merupakan penunjuk biologi yang paling baik untuk logam berat pencemar dalam udara. Kesimpulannya, lumut tempatan mempunyai potensi sebagai penunjuk biologi yang baik bagi logam berat di kawasan tanah tinggi tropika dan boleh digunakan untuk pemonitoran jangka panjang.

Kata kunci: *Lumut jati, pencemaran udara, logam berat, penunjuk biologi.*

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LIST OF ABBREVIATION AND SYMBOL

	Measurement Units
-	Minus, negative or to
>	More/higher/greater than
<	Less/lower than
°C	Degree celcius
%	Percent
µg/g	Micrograms per gram
$\mu g\ g^{-1}$	Micrograms per gram
µg/mL	Micrograms per millilitre
$\mu g/m^3$	Micrograms per cubic metre
A	Absorbance
Alt.	Altitude
cm	Centimetre
dw	Dry weight
E	East
EF	Enrichment factor
g	Gram
kg	kilogram
km	Kilometre
L	Litre
M	Mole
m	Metre
mg	Milligram
mL	Millilitre

N	North
nm	Nanometre
mm	millimetre
PM_{10}	Particulate matter
ppm	Part per million
rpm	Rotations per minute
TCCs	Total chlorophyll contents
V	Volume
AAS	Atomic absorption spectrometry
	Chemical Compounds
Ag	Silver
Al	Aluminium
As	Arsenic
Au	Gold
B	Boron
Ba	Barium
Bi	Bismuth
$(CH_3)_3Pb$	Trimethyl lead
$(CH_3CH_2)_4Pb$	Tetraethyl lead
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Ca	Calcium
Cd	Cadmium
Co	Cobalt
CO_2	Carbon dioxide

CO	Carbon monoxide
Cr	Chromium
Cu	Copper
DMSO	Dimethyl sulfoxide
Fe	Iron
GSH	Glutathione-S-transferase
HCB	Hexachlorobenzene
HCHs	Hexachlorocyclohexanes
Hg	Mercury
HNO_3	Acid nitric
K	Potassium
Li	Lithium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
NO	Nitrogen oxide
O_3	Ozone
OC	Organochlorine compounds
P	Phosphorus
PAH	Poly-aromatic hydrocarbon
Pb	Lead
PCBs	Polychlorobiphenyls
PCBz	Pentachlorobenzene
PCCDs	Polychlorinated dibenzodioxins

PCDFs	Polychlorinated dibenzofurans
Pd	Palladium
Pt	Platinum
PVC	Polyvinyl chloride
Rh	Rhodium
SO_2	Sulphur dioxide
TSP	Total suspended particulate
UV	Ultraviolet
	Elementals
S	Sulphur
Sb	Antimony
Se	Selenium
Si	Silicon
Sr	Strontium
Th	Thorium
Ti	Titanium
Tl	Thallium
U	Uranium
V	Vanadium
Zn	Zinc
	Moss Species
Bp	*Brachythecium plumosum*
Ba	*Bruetelia arundinifolia*
Hd	*Hypnodendron dendroides*
Lj	*Leucobryum javense*

Ls	*Leucobryum sumatranum*
Sc	*Sphagnum cuspidatum*
Ss	*Sphagnum sericeum*
Sr	*Spiridens reinwardtii*
Tc	*Trismegistia calderensis*
Tp	*Trismegistia panduriformis*
	Monitoring Sites
L1	Monitoring Site 1
L2	Monitoring Site 2
L3	Monitoring Site 3
L4	Monitoring Site 4
L5	Monitoring Site 5
L6	Monitoring Site 6
L7	Monitoring Site 7
L8	Monitoring Site 8
L9	Monitoring Site 9

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CHAPTER I

INTRODUCTION

1.1 Introduction

Moss (Bryophyta), have been widely used as biomonitoring organisms in the last four decades. There are a large number of studies carried out in this field that support its applicability (Siebert *et al.*, 1996; Bruns *et al.*, 1997; Halleraker *et al.*, 1998; Szczepaniak & Biziuk, 2003; Schintu *et al.*, 2005; Pesch & Schroeder, 2006). Studies on mosses in relation to environmental pollution started since 1970's. It was advocated by two Swedish scientists, Åke Ruehling, and Germund Tylor ,in the late 1960's (Pesch & Schroeder, 2006). They used mosses as biological indicators for heavy metal accumulation from ambient air.

Bryophytes are the most abundant plants on the lithosphere. These non-vascular plants are small in size, vary in shape, compact, complex, evergreen, and have the ability to synthesize their own food (Giordano *et al.*, 2005; Uno *et al.*, 2001). Moss has the ability to reflect the actual condition of air quality in environmental study, water quality in hydrology or in limnological studies and soil quality in forestry or in agriculture (Frahm *et al.,* 1990). Its ability to grow on mineralised substrates such as copper, lead or zinc makes it suitable to show the accumulation rates and mineral depositions (Frahm *et al.*, 1990).

Mosses have several advantages to with higher plants as a biomonitoring organism (Čerburnis & Valiulis, 1999; Giordano *et al.*, 2005; Adamo *et al.*, 2008). The lack of a thick cuticle promotes the migration of heavy metals and other elements to the free cation exchange sites located on the walls of the cells in mosses (Poikolainen *et al.*, 2004). The use of mosses as biomonitors is a very convenient method for determining the total levels of atmospheric deposition of contaminants (Fernández *et al.*, 2000; Cesa *et al.*, 2006).

Biological monitoring can be carried out in two different manners, active monitoring and passive monitoring (Szczepaniak & Biziuk, 2003). Active monitoring involves the exposure of well-defined species under controlled conditions, while passive monitoring refers to the observation or chemical analysis of indigenous plants (Cao *et al.*, 2009). Mosses have been used as active and passive biomonitors to estimate the deposition of contaminants in polluted areas (Fernández *et al.*, 2000). Transplant techniques have been used in active biomonitoring to estimate and assess the impact of metals associated with ambient particles on mosses at contaminated areas (Figueira & Ribeiro, 2005; Adamo *et al.*, 2008).

The moss bag technique is one of the effective active biomonitoring methods. This technique was originally introduced by Goodman and Robert (1971) and was later modified by other researchers. This technique is useful because it helps to keep the humidity of the moss samples stable and prevent from drying (Szczepaniak & Biziuk, 2003). This method proved that mosses are effective bioindicators of atmospheric pollutants as they are able to take up nutrients and pollutants directly from the atmosphere due to lack of root systems (Lim *et al.*, 2006; Tremper *et al.*, 2004; Tretiach *et al.*, 2007). The transplant technique is useful and effective in biomonitoring studies because it can help to determine the changes in environmental quality. The element accumulations in moss materials can be compared between the control population and transplanted material in exposed sites.

There are a wide variety of sources that contribute to trace metals in the atmosphere, such as combustion processes, metal industries and mining. Traffic plays the major local source of metal pollution, especially in urban areas (Tremper *et al.*, 2004). Mosses actually require small amounts of heavy metals for their metabolic functioning (Poikolainen *et al.*, 2004). However, they still can accumulate heavy metals more than the amount required. The accumulation of metals in mosses depends on many factors such as the availability of the elements, the characteristics of the mosses (such as species, age, state of health and type of reproduction), temperature, moisture availability and substratum characteristics

(Conti & Cecchetti, 2001). These factors reflect the environmental conditions of the areas as a result of the equilibrium process of biota compound intake or discharge from and into the surrounding environment.

Atmospheric pollution has become a major concern in many developing countries. Scientists from China, Italy, India, Spain, Romania and USA have carried out many studies on atmospheric contamination but most of them were limited by problems of high costs and the difficulty of carrying out extensive sampling, in terms of both time and space (Cao *et al.*, 2009). As an alternative way, many of these scientists have started to use an indirect monitoring method using organims that are identified as biological accumulators (Fernández *et al.*, 2004; Cao *et al.*, 2009; Sharma, 2009). Biomonitoring studies utilizing mosses have been conducted in the western hemisphere but there are very limited studies reported from Asian region. One example is a study by Lim *et al.* (2006) in Singapore on persistent organic pollutants using mosses as bioindicators of atmospheric pollution. It showed that mosses' physiology enables the plants to actively uptake water, nutrients and pollutants from the air.

Thus far, no monitoring study using mosses have been reported for Sabah. However, several preliminary studies have been done in Kota Kinabalu using lowland moss species to study the potential of local plants as biomonitors for atmospheric heavy metals pollution (Lee, 2006; Khairul, 2007; Masundang, 2007). Sabah has very high diversity of mosses, with 582 species (Suleiman *et al.,* 2006). A big percentage of these mosses are abundant in highlands, such as Mount Kinabalu. No attempt have been done to study the potential of native highland mosses as biomonitors in Malaysia. Many mountains in the country are popular tourists destination. This has caused air pollution resulting from heavy vehicle traffic. Non expensive biomonitoring using native plants can be adopted in management strategies for protected areas, such as Kinabalu Park.

1.2 Objectives

The objectives of this study are outlined as follows:

i) To determine the heavy metal concentrations in selected mosses after an exposure period at monitoring sites.

ii) To compare the chlorophyll content of selected mosses before and after exposure.

iii) To determine the relationship between heavy metal elements, exposure periods, monitoring sites and rate of bioaccumulation.

iv) To determine the accumulation capacity of the selected mosses.

v) To determine which moss species are good bioindicator for determining atmospheric heavy metal pollution based on its accumulation capacity.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Bryophytes are well recognised as biological indicators of atmospheric pollution in biological monitoring studies all over the world (Onianwa, 1988; Vasconcelos and Tavares, 1998; Carballeira *et al.*, 2002; Aboal *et al.*, 2007; Adamo *et al.*, 2008). Many developing countries and other countries that concerns of their environmental health carried out numerous studies using bryophytes to evaluate their atmospheric conditions (Table 2.1).

Table 2.1: Studies Using Bryophytes Around the World

No.	Country	Bryophytes	Reference (Year)
1.	Austria	*Abietinella abietina*	Zechmeister *et al.*, 2003
		Hylocomium splendens, *Pleurozium schreberi* and *Scleropodium purum*	Zechmeister *et al.*, 2004
		Abietinella abietina, *Hylocomium splendens*, *Hypnum cupressiforme*, *Pleurozium schreberi* and *Scleropodium purum*	Zechmeister *et al.*, 2005
		Abietinella abietina, *Hylocomium splendens*, *Pleurozium schreberi* and *Scleropodium purum*	Zechmeister *et al.*, 2008
		Meilichhoferia elongata and *Physcomitrella patens*	Sassmann *et al.*, 2010
2.	Bulgaria	*Hypnum cupressiforme*	Coskun *et al.*, 2009
3.	Canada	*Sphagnum* peat moss	Champagne and Li, 2009
4.	China	*Hypnum plumaeforme*	Lee *et al.*, 2005
		Hypnum revolutum	Bi *et al.*, 2006