ASSESSMENT OF MOSS SPECIES AS BIOMONITORS OF ATMOSPHERIC POLLUTANTS IN SOME TOWNS OF NORTH-WESTERN NIGERIA

BY

SA'IDU ABDULLAHI

DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,AHMADU BELLO UNIVERSITY, ZARIAIN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARDOF AMASTER OF SCIENCE DEGREE IN BOTANY

DEPARTMENT OF BIOLOGICAL SCIENCES,

FACULTY OF SCIENCE,

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

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DECLARATION

I declare that the work in this Thesis entitled "ASSESSMENT OF MOSS SPECIES AS BIOMONITORS OF ATMOSPHERIC POLLUTANTS IN SOME TOWNS OF NORTH-WEST NIGERIA" has been carried out by me in the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Sa'idu ABDULLAHI	______________________	________________
	Signature	Date

CERTIFICATION

This thesis entitled "ASSESSMENT OF MOSS SPECIES AS BIOMONITORS OF ATMOSPHERIC POLLUTANTS IN SOME TOWNS OF NORTH-WEST NIGERIA" by Sa'idu ABDULLAHI meets the regulations governing the award of the degree of Master of Botany of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. S. P. Bako ____________________ __________________
Chairman, Supervisory Committee Signature Date
Department of Biological Sciences,
Ahmadu Bello University, Zaria.

Dr B. Y. Abubakar ____________________ __________________
Member, Supervisory Committee Signature Date
Department of Biological Sciences,
Ahmadu Bello University, Zaria.

Prof. A. K. Adamu ____________________ __________________
Head of Department Signature Date
Department of Biological Sciences,
Ahmadu Bello University, Zaria.

Prof. A. Z. Hassan ____________________ __________________
Dean, School of Postgraduate Studies Signature Date
Ahmadu Bello University, Zaria.

DEDICATION

I dedicate this work to my late Father.

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ABSTRACT

The development of industry, urbanization and increase in vehicular emissions in Nigeria has resulted in the contamination of the environment by heavy metals and other pollutants. Air borne heavy metals and other pollutants (including NO_X and SO_2) enter the ecosystem where they circulate and, depending on their concentration and toxicity, pose a greater or smaller threat to the components of these ecosystems.Thus, this work is designed tostudy the use of moss species as biomonitors of air pollutants in some towns of North-West Nigeria.Moss sampling was carried out during the dry and wet seasons from various substrates. The distribution/occurrence of the plants on the various substrates was calculated.Foreign materials adhering to the moss samples were removed washed with double-distilled water and oven dried at 60° C for 48 hours. They were then ground in a porcelain mortar to obtain fine particles and stored in polythene bags ready for analyses.Heavy metal contents (Co, Ni, Cu, Cd, Pb, Cr, and Zn) in the samples were determined using Fast Sequential Atomic Absorption Spectrometer (Varian AAS 240FS).Determination of NO_3 and NO_2 were done by steam distillation while that of SO_2 was doneturbidimetrically.ANOVA was used to test for differences in individual concentrations in the species and the different locations, Duncan's Multiple Range Test (DMRT) was used to separate the means in case they are significantly different and T-test used to determine seasonal variability of mean levels of pollutants in the moss species.A total of about 144 samples were collected throughout, representing eight (8) different species of moss with*Hyophilacrenulata*having the highest percentage of occurrence.Kano was having the highest mean concentrations of 4 of the 7 heavy metals analyzed which are Cu, Cd, Cr and Pb.Kano had the highest concentrations of almost all the major pollutants, while Ringim had the least of the majority.Ni, Cu, Cd, Cr and Co showed relatively higher accumulation values in the wet season than the dry season, while Pb and Zn had higher values in the dry season but statistically not significant in all cases.From the study, it can be said that out of the different mosses studied, three species*Bryumcoronatum, Fissidensgrandifolius* and *Hyophilacrenulata* were precise and useful bioindicators/biomonitors of heavy metals in the studied locations. Among them *B.coronatum*is the good accumulator of majorityof themetals. SO_2 accumulation was better up in *Barbulalambarenensis*and *Erpodiumpobeguini*, while *Fissidensgrandifolius*was better in accumulating NO_2 and NO_3.There is no significant variation in accumulation of all the studied pollutants in the moss plants between wet and dry seasons. Heavy metals (Co, Ni, Cu, Cd, Pb, Cr, and Zn) as well as SO_2, NO_2 and NO_3 were present in the environments of all the study locations, at the time of sampling. The results provide baseline information for regional atmospheric pollution monitoring using moss species as effective biomonitors in the studied locations.

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CHAPTER ONE

1.0 INTRODUCTION

Air pollution has become a matter of global concern, particularly in developing countries, mainly due to various anthropogenic activities. Significant contribution of air pollution to the diminished health status of the exposed human populations, forest decline, loss of agricultural productivity, etc., has been a cause of increasing public concern throughout the world (Smodis, 2003). According to Markert *et al.* (2003), the environment through human activity has been confronted with totally new substances that did not previously exist (xenobiotics, many radionuclides) and potentially harmful substances released in quantities unthinkable in the past (heavy metals, natural radionuclides). Therefore, it has become a topic of intense scientific, governmental and also industrial interest.

The quick development of industry, motor transport and urbanization caused a dramatic increase in dust emissions containing heavy metals. Ecologists focused their attention on threats posed by heavy metals and other atmospheric pollutants to the biotic and a-biotic environment. They began to look for sensitive and cheap biological methods for assessing the environmental level of heavy metals, most especially the toxic ones like Cd, Pb and Hg (Zechmeister *et al*., 2003). The usefulness of bio-monitoring as a technique to investigate trace element atmospheric pollution has been reported by many authors (Markert *et al*., 2003; Smodis, 2003; Chakrabortty and Paratkar, 2006).

The Clean Air Act of the United State Environmental Protection Agency recognized six common air pollutants (US-EPA, 2012). These commonly found air pollutants (also known as "criteria pollutants") are found all over the United States. They are particle pollution (often referred to as particulate matter), ground-level ozone, carbon monoxide, sulfur dioxides, nitrogen oxides, and lead. These pollutants can harm human health and the environment, and cause property damage. Of the six pollutants, particle pollution and ground-level ozone are the most widespread health threats (US-EPA, 2012). The US-EPA calls these pollutants "criteria" air pollutants because it regulates them by developing human health-based and/or environmentally-based criteria (science-based guidelines) for setting permissible levels. The set of limits based on human health is called primary standards. Another set of limits intended to prevent environmental and property damage is called secondary standards. Of immediate concern is the release of various heavy metals like Pb, Hg, Cd, Ni, Zn etc. due to different anthropogenic activities. Thus continuous monitoring of air quality is pre-requisite. This can be accomplished according to Harinder (2006) either by the construction of computer models, mechanical collectors or by the employment of bio-indicators/ bio-monitors.

There are two conceptual approaches for collecting samples relevant to air and atmospheric deposition related pollution studies according to IAEA (2003): (1) the direct collection of APM, precipitation and total deposit, and (2) the use of air pollution biomonitors. The first approach is aimed at quantitative surveys at local, short-range, medium-range or global transport of pollutants, including health-related studies when collecting size-fractionated APM. It requires continuous sampling on a long-term basis at a large number of sites, in order to ensure the temporal and spatial representativeness of measurements. The application of such direct measurements on a large scale is extremely costly and person-power intensive. Furthermore, it is not possible, due to logistic problems, to install instrumental equipment at all needed locations. Therefore, the second approach is considered as a non-expensive, yet reliable means of air quality status assessment in a country or a region. It was shown by Zechmeister *et al.* (2003) that Bryophytes can be used as indicators and monitors in terrestrial habitats for heavy metals, Nitrogen compounds, Sulphur compounds, toxic organic compounds and radionuclides.

Biological monitoring has been described as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program (Mathew *et al*., 1982). Markert *et al*. (2003) defines bioindicator as an organism that contains information on the quality of the environment; and a biomonitor on the other hand, as organism that contains information on the quantitative aspects of the quality of the environment. Biomonitoring of air pollution is emerging as a potentially effective and more economical alternative performing by direct ambient air measurements. This is especially relevant for monitoring large areas (Ruhling and Tyler, 1968; Onianwa *et al*., 1986).

The suitability of Bryophytes for the indication and monitoring of atmospheric heavy metal deposition is based on their accumulation of the metals which depends on their morphological and physiological properties. These include presence of rhizoids in place of real roots so that they cannot take enough nutrients from the soil; nutrient uptake from the atmosphere promoted by weakly developed cuticle and one cell thick leaves; large surface to weight ratio improving absorption; slow growth rate which enables them accumulate pollutants over time; perenniality; wide distribution and ease of sampling (Zechmeister *et al.*, 2003; Chakrabortty and Paratkar, 2006). It is possible to make deductions as to the presence of various air pollutants and their levels on the basis of the occurrence and chemical constitution of epiphytic plants such as lichens and bryophytes (Pyatt *et al*., 1999; IAEA, 2000; IAEA, 2003).

The use of mosses as indicators of atmospheric pollution by heavy metals is now well established. Apart from the monitoring of emissions from local hot spots such as the vicinities of industrial complexes, mines and highways (Onianwa and Ajayi, 1987; Fatoba and Oduekun, 2004; Dymitrova, 2009; Ekpo *et al*., 2012), mosses have also been used for extensive regional surveys in various parts of the world (IAEA, 2000; Chen *et al*., 2010; Ekpo *et al*., 2012). This technique has proved rapid and reliable in identifying metal emission and deposition gradients over vast areas.

Mosses are abundant in Nigeria and an earlier survey (Onianwa and Egunyomi, 1983) has shown the local species to be suitable for biomonitoring atmospheric heavy metal pollution. Onianwa and Ajayi (1987) have surveyed the South West region of Nigeria using various species of mosses collected from forest sites and inhabited sites in the region. Other studies in the use of mosses as environmental biomonitors in parts of Nigeria have been reported (Kakulu, 1993; Fatoba and Oduekun, 2004; Bako *et al*., 2008; Adebiyi and Oyedeji, 2012; Ekpo *et al*., 2012). However, use of biomonitors in monitoring environmental quality in the West African region is limited (Bako *et al*., 2008).

In North West region of Nigeria, it is suspected that the buildup of air pollutants may be anticipated as a result of obvious polluting sources such as agricultural practices, urbanization, rapid industrialization and the resulting heavy traffic. There is therefore a need to monitor pollutants and determine their concentration in the region. Thus, this work is designed to identify the heavy metals, nitrogen oxides and sulfur dioxide, and determine their concentration in the North West region of Nigeria using moss species as biomonitors. The result is expected to provide base line information for adequate pollution monitoring and control in the region. It would also test the effectiveness of the use of moss plants for pollution monitoring.

1.1 Statement of the Research Problem

The development of industry, urbanization and increase in vehicular emissions in Nigeria has resulted in the contamination of the environment by heavy metals and other pollutants. Air borne heavy metals and other pollutants (including NO_x and SO_2) enter the ecosystem where they circulate and, depending on their concentration and toxicity, pose a greater or smaller threat to the components of these ecosystems. The accumulation of heavy metals in the soil and living organisms has damaging effects (Herpin *et al*., 1996).

Environmental pollution has been studied to cause severe illness and sudden death in human beings for many centuries. Different diseases and subsequent deaths have been documented in Nigeria. For example, Zamfara lead poisoning that claimed the lives of over 500 children, and left thousands in severe health situations in 2010, is the worst of its kind in the global record of the year according to Galadima *et al*. (2011). It can be air, water or land and can result from mining, automobile exhaust, agricultural and industrial activities among others. Heavy metals were since classified among major causes (Galadima *et al*., 2011). In general, developing countries experience higher levels of traffic related pollution compared to developed countries due to the lack of pollution control measures.

Controlling anthropogenic air pollutants is a very complex problem where sources and emissions have to be managed and monitored and economic aspects have to be integrated (Sloof, 1993). Most air pollution studies are based on atmospheric aerosols collected on particulate matter filters. There is lack of sufficient, sensitive and inexpensive techniques that permit the simultaneous measurement of many air contaminants (Pucket, 1988). In Nigeria, it is difficult to use air samplers in most areas due to erratic power supply.

1.2 Justification

Use of moss species as biomonitors has proved rapid and reliable in identifying metal emission and deposition gradients over vast areas. It is comparatively much cheaper than the use of standard automatic samplers which usually involve prohibitive costs. This makes moss as biomonitors particularly suitable for use in developing countries such as Nigeria.

It was reported by Wolterbeek (2003) that the relative ease of sampling, the absence of any need for complicated and expensive technical equipment, and the accumulative and time-integrative behavior of the monitor organisms make that moss biomonitoring of atmospheric trace elements will be continued for the foreseeable future especially on larger scales.

According to Borrego *et al.* (2003), monitoring of biological matter such as soils, vegetables, and animal tissues, represents also an appropriate way to determine the exposure paths of the human population to atmospheric pollutants. These studies require a good description of the atmospheric pollutants deposition process and provide a cost-effective mechanism to perform a long-term sampling. Bryophytes are very resistant against a series of substances which are highly toxic for other plants (e.g. heavy metals, radionucleides, and various toxic organic compounds). As a consequence of their nutrient cycling and uptake mechanisms they even tend to accumulate these pollutants (Zechmeister *et al*., 2003).

Moreover, mosses can be stored for several years without noticeable deterioration and old specimens can easily be chemically analyzed (Harinder, 2006). Therefore, a comparison between fresh and herbarium specimens can be performed. It is still noticeable that both herbarium and fresh specimens should originate from the same locality and provides a good insight of the past and present heavy metal burden at the research area. Thus bryophytes can serve as “Environmental Specimen Banks” (Harinder, 2006).

One can also predict the suitable moss species that may be used as a biomonitor for a single trace element, or a group of trace elements. It is very difficult for any other approach to obtain such a detailed picture of variations in time and space at a reasonable cost (Chakrabortty and Paratkar, 2006).

The usefulness of biomonitoring as a technique to investigate trace element atmospheric pollution has been reported by many authors worldwide (Ruhling and Tyler, 1968; Pyatt *et al*., 1999; Markert *et al*., 2003; Smodis, 2003; Chakrabortty and Paratkar, 2006; Chen *et al*., 2010). Most of the atmospheric trace metal pollution studies in Nigeria have been carried out in the Southern part of the country (Onianwa *et al*., 1986; Onianwa and Ajayi, 1987; Fatoba and Oduekun, 2004; Adebiyi and Oyedeji, 2012; Ekpo *et al*., 2012). Very few were carried out in the Northern part of the country (Kakulu, 1993; Bako *et al*., 2008; Bantam, 2011). Also, In Nigeria much attention is given on general industrial pollution and pollution in oil industries, with little reference on damage of pollution caused by mobile transportation sources of air pollution (Faboye, 1997; Magbabeola, 2001).

The data base for monitoring the environmental quality and for setting quality criteria particular to the Nigerian environment, with reference to tropical bryophyte and lichen species is still rather scanty. Information on the impact of environmental changes on the ecosystem and biodiversity are needed to provide policy makers with appropriate information to regulate environmental pollution (Erisman *et al.*, 2002). This form of study becomes pertinent in the present climate change and it's attending consequences.

1.3 Aim of the Study

The aim of this work is to study the use of moss species as biomonitors of air pollutants in some towns of North-West Nigeria.

1.4 Objectives of the Study

The objectives of the study are to

1. identify the common moss species found in the study areas
2. determine the concentration of atmospheric heavy metals in the moss species inhabiting the various locations
3. determine the concentration of oxides of nitrogen and sulphur dioxide in the moss species
4. determine the seasonal variability of the levels of the atmospheric pollutants.

1.5 Research Hypotheses

1. There is no significant difference with respect to moss species found in the study areas.
2. There is no significant difference in the concentration of atmospheric heavy metals among moss species of the various locations.
3. There is no significant difference in the concentration of atmospheric oxides of nitrogen and sulphurdioxide among moss species found in the various locations.
4. There is no significant difference in the concentration of atmospheric pollutants between dry and wet seasons.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Bryophytes

Bryophyte is a traditional name used to refer to all embryophytes (land plants) that do not have true stems, leaves, roots and vascular tissues. Bryophytes are regarded as transitional between aquatic plants like algae and higher land plants like trees. In the 1600's, Jung considered mosses to be aborted plant fetuses (Crum, 2001). Today, they occupy a position within the plant kingdom and may even be considered to have their own subkingdom. A broad consensus among systematists has recently emerged that bryophytes as a whole are not a natural or monophyletic group (with common ancestors), but are paraphyletic/polyphyletic (with several ancestral lines).

However, the name is convenient and remains in use as a collective term for mosses, liverworts and hornworts, although each of the three extant (living) groups is monophyletic (Konrat *et al*., 2010). The three lineages are Marchantiophyta (liverworts), Bryophyta (mosses) and Anthocerotophyta (hornworts). Most bryophytes are either liverworts or mosses. Liverworts grow horizontally, and are flattened or leafy, whereas mosses have an upright stalk with spirally arranged leaf-like structures (Saxena and Harinder, 2004). The pleurocarpous mosses (carpet-forming) are characterized by extensive branching and lateral sporophyte placement, compared to the terminal sporophytes in acrocarpous (erect) mosses.

Among the world of plants, the bryophytes are the second largest group, exceeded only by the Magnoliophyta – the flowering plants (350,000 species). Comprised of 15,000

(Gradstein *et al*., 2001) – 25,000 species (Crum, 2001), they occur on every continent and in every location habitable by photosynthetic plants. This group of plants which many plant scientists refer to as the "amphibians" of the plant kingdom, live on land and in damp places but breed only in the presence of water. The life history of bryophytes involves an alternation between sporophytic and gametophytic generations that differ in form and function. The actual plant is represented by the gametophytic generation, which is the most evolved haploid generation in the whole plant kingdom (Zechmeister *et al*., 2003).

Most of the bryophytes are ectohydric species, which means that most of the species receive water as well as mineral nutrients predominantly by atmospheric depositions (Zechmeister *et al*., 2003). They are well adapted to this strategy since they have no or only very small vacuoles, and beside some surface wax structures (e.g. papillae) there is no continuous water-repellent cuticle. Bryophytes seem all the more elaborate because of their small size. Some bryophytes are only a few millimeters tall and have but few leaves, as in the mosses *Ephemeropsis* and *Viridivellus pulchellum* (Crum, 2001). The more common *Buxbaumia* has a large capsule on a thick stalk, but only a few special leaves protect the archegonia; the plant depends on its protonema (and later the capsule) to provide its photosynthate. At the other end of the scale, the moss *Polytrichum commune* can attain more than half a meter height in the center of a hummock and *Dawsonia superba* can be up to 70 cm tall with leaves of 35 mm length (Crum, 2001) and be self-supporting. *Fontinalis* species, supported by their water habitat, can be 2 m in length. Bryophytes, especially mosses are found in almost every habitat that supports life and their ecological role is significant (Saxena and Harinder, 2004).

2.2 Concept of air Pollution/Pollutants

Air pollution can be defined as the introduction of chemicals, particulates, or biological materials/substances into the atmosphere that cause discomfort, disease, or death to humans, damage other living organisms such as food crops, or damage the natural or built environment. A substance in the air that can be harmful to humans and the environment is known as an air pollutant. The main components include sulphur dioxide, airborne particulate matter (APM), carbon monoxide, reactive hydrocarbon compounds, nitrogen oxides, and ozone (Smodis and Bleise, 2003). Among them, heavy metals and other toxic elements, mostly associated with APM, represent an important group to be considered. Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546. The definition stated here includes some heavy elements from group III to V in the periodic (Galadima and Garba, 2012). After emission, the pollutants are subjected to physical, chemical, and photochemical transformations, which ultimately decide their fate and atmospheric concentrations (Smodis and Bleise, 2003).

A wide array of air pollutants including particulates, liquids, and gasses are being emitted both from natural and anthropogenic sources. Natural sources include volcanic emissions, accidental fires in forests and on prairies, dust storms, soil particles, salt particles emitted from the oceans, and various products given off by plants. Anthropogenic sources of air pollution comprise emissions due to industrial activities (e.g. manufacturing products from raw materials, industries that convert products to other products, etc.), power generation (e.g. fossil fuel combustion), traffic, agriculture, waste incineration, residential heating, and many others (Smodis, 2003).

Many studies have documented adverse health effects associated with high concentrations of transport-related pollutants. Nitrogen oxides and sulfur oxides, for example, are associated with immune system impairment, exacerbation of asthma and chronic respiratory diseases, reduced lung function, and cardiovascular disease (Schwela, 2000). Further oxidation of SO_2, usually in the presence of a catalyst such as NO_2, forms H_2SO_4, and thus acid rain (AHA, 2010). This is one of the causes for concern over the environmental impact of the use of these fuels as power sources. Sulphur Dioxide is emitted directly into the atmosphere and can remain suspended for days allowing for wide distribution of the pollutant (Adoki, 2012). Sulphur dioxide in the air is hazardous to vegetation, and its High emission level is due to some extent from automobile emissions (Adoki, 2012).

All high-temperature processes produce NO_3 which is then oxidized further to NO_2 in the ambient air. At ambient concentration levels, NO_2 is an irritating gas that may constrict the airways of asthmatics (Adoki, 2012). Airborne heavy metals enter the ecosystems where they circulate and, depending on their concentration and toxicity, pose a greater or smaller threat to the components of these ecosystems. The accumulation of heavy metals in the soil and living organisms may have a damaging effect on the environment (Lieth and Markert, 1990; Markert, 1993; Herpin *et al*., 1996).

2.3 Concept of Bioindication/Biomonitoring of the Environment

According to Markert *et al*. (1997; 1999), A bioindicator is an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment). A biomonitor, on the other hand, is an organism (or a part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment. A biomonitor is always a bioindicator as well, but a bioindicator does not necessarily meet the requirements for a biomonitor. Wolterbeek (2003) defined biomonitoring as the use of bio-organisms/materials to obtain (quantitative) information on certain characteristics of the biosphere. The relevant information in biomonitoring (e.g. using plants or animals) is commonly deduced from either changes in the behaviour of the monitor organism (impact: species composition and/or richness, physiological and/or ecological performance, morphology) or from the concentrations of specific substances in the monitor tissues (Wolterbeek, 2003).

Certain types of organisms integrate pollution over time, reducing the need for continuous chemical monitoring, thus avoiding the difficulty of interpreting “snapshot” measurements and offering the potential of retrospective monitoring (IAEA, 2003). By observing and measuring the changes in an appropriately selected organism, a conclusion as to the kind of pollution, its source, and its intensity can be drawn. With proper selection of organisms, the general advantage of the biomonitoring approach is related primarily to the permanent and common occurrence of the organism in the field, even in remote areas, the ease of sampling, and the absence of any necessary expensive technical equipment (Wolterbeek, 2003). Plant bio-monitoring is an attractive alternative method to detect and to monitor particles present in the atmosphere, owing to the fact that they are well collected by leaf surfaces due to their particular properties (Garrec *et al*., 2003). Mosses and lichens as well, are considered as the most appropriate plant material to study the atmospheric deposition of heavy metals, yet pine needles, tree bark, mushrooms have also been used to monitor persistent toxic pollutants including heavy metals (Harinder, 2006).

Active bioindication (biomonitoring) is when bioindicators (biomonitors) bred in laboratories are exposed in a standardized form in the field for a defined period of time (Markert *et al*., 2003). At the end of this exposure time the reactions provoked are recorded or the xenobiotics taken up by the organism are analyzed. In the case of passive biomonitoring, organisms already occurring naturally in the ecosystem are examined for their reactions. The advantage of active monitoring is however, that it is performed in those study areas where naturally growing monitors are absent e.g. in heavily polluted areas (Harinder, 2006).

A classification of organisms (or communities of these) according to their "mode of action" as explained by Markert *et al*. (2003) is as follows: Accumulation indicators/monitors are organisms that accumulate one or more elements and/or compounds from their environment; Effect or impact indicators/monitors are organisms that demonstrate specific or unspecific effects in response to exposure to a certain element or compound or a number of substances. Such effects may include changes in their morphological, histological or cellular structure, their metabolic-biochemical processes, their behavior or their population structure. In general the term "reaction indicator" also includes accumulation indicators/monitors and effect or impact indicators/monitors as described above.

Major advantages of biomonitoing include: a) No long term use of expensive sampling equipment is required. b) Sampling of organisms used as biological monitors is generally easier. c) The concentrations in the monitor organisms are higher than the system to be monitored. This improves the accuracy of measurements. d) Most organisms reflect external conditions averaged over certain periods of time. This becomes important when monitoring levels change rapidly with time. Chakrabortty and Paratkar (2006) mentioned a number of selection criteria of the monitors for trace element air pollution. These include that:

- The organism has to be common in the area of interest.
- It has to be available for sampling during all seasons; if not, then some simple special devices have to be developed to grow it in all seasons.
- The monitor should be tolerant of pollutants at the relevant levels.
- Element uptake should be independent of local conditions.
- The biological variation of the organism should be limited.
- The accumulated concentration levels must be measurable by routine analytical techniques.
- Absence of appreciable amounts of element uptake from sources other than the atmosphere.
- Physiological mechanisms for uptake of elements should be known to facilitate interpretation of the results.

- The biomonitor should average the elemental concentrations over a period of time as a result of integrated exposure.
- The organism should have low background concentrations of these elements.
- The sampling method and protocol for sample preparation for measurement should be simple and quick.

Suitable biomonitors, which meet the requirements, make continuous monitoring and even retrospective monitoring of air pollution possible at relatively low cost. When information on time-averaged trace-element concentrations at specific sites in the environment is the aim, the use of such non-mobile monitors is preferred (Chakrabortty and Paratkar, 2006). According to Borrego *et al* (2003), monitoring of biological matter such as soils, vegetables, and animal tissues, represents also an appropriate way to determine the exposure paths of the human population to atmospheric pollutants. These studies require a good description of the atmospheric pollutants deposition process and provide a cost-effective mechanism to perform a long-term sampling.

One of the most important challenges in biomonitoring studies is to establish standardized protocol for sampling, sample preparation, elemental analysis in order to obtain comparable results on at least a regional scale. It is possible to collect mosses in selected areas ranging from pollution-free background regions to highly polluted regions. One can also predict the suitable moss species that may be used as a biomonitor for a single trace element, or a group of trace elements. It is very difficult for any other approach to obtain such a detailed picture of variations in time and space at a reasonable cost (Chakrabortty and Paratkar, 2006).

2.4 Use of Moss Species as Biomonitors of Atmospheric Pollution

Mosses have been used for evaluating the present state of environmental contamination in both extensive and small areas and also the pollution level in the past (Zechmeister *et al*., 2003). Many European countries have used mosses since the beginning of 1960s in national and multinational surveys of atmospheric metal deposition (Ruhling, 1994). Since Ruhling and Tyler's pioneer research in 1968 using moss as sensitive bioindicators of heavy metal contamination, use of terrestrial mosses for biomonitoring atmospheric pollution has been widely applied (e. g Onianwa *et al.*, 1986; Kakulu, 1993; Shakya *et al*., 2001; Fatoba and Oduekun, 2004; Dymytrova, 2009; Chen *et al*., 2010; Ekpo *et al*., 2012;). *Hylocomium splendens, Pleurozium schreberi* and *Hypnum cupressiforme* were commonly employed as bioindicators of atmospheric pollution in Europe and the North America (Bargagli *et al* 1995; Poikolainen *et al.*, 2004).

However, due to their limited distribution, some other mosses were selected for the investigation such as *Bryum radiculosum, Aloina aloides, Tortella flarovirens, Scleropodium purum and Polytrichum formosum* in the Czech and Slovak republics (Markert *et al*., 1996). The moss species were used to obtain information on the regional deposition of heavy metals, changes in the deposition patterns the long distance spread of emissions and local emission sources (Chakrabortty and Paratkar, 2006). Kansen and Vegra (1991) made use of earthworm and moths in a comparative study of the capability of some plants and animals in assessing air borne Chromium and Nickel dust near a ferro-chrome and stainless steel works in Finland. They observed that mosses and epiphytic lichens are the most effective bioindicators.

Zechmeister *et al*., (2003) mentioned certain techniques for the assessment of heavy metal levels of the environment which include indigenous moss technique, live moss transplanting and moss bag technique. Moss technique was first used for large scale monitoring purposes in the scandinavian countries- Sweden, Norway, Finland and Denmark (Ruhling *et al*., 1987). The results of studies conducted within the framework of European joint moss research in 1990-1995 using three moss species- *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.)B. S. G. and *Hypnum cupressiforme* agg. confirmed regional variations in the deposition of heavy metals in Europe, as mentioned in earlier decades (Zechmeister *et al*., 2003). They showed a sharply decreasing gradient from Central Europe to northern Scandinavia for Cd, Pb and V. A weaker gradient was obtained for Cr, Cu, Fe, Ni and Zn, and particularly for Ni and Cu in the far north, in the western part of the Kola Peninsula where there are big smelting plants. The moss technique is also very useful for assessing environmental contamination on a local scale, in the neighborhood of industrial plants, in towns and in the vicinity of highways (Ruhling and Tyler, 1968).

The transplantation technique consists in transplanting living mosses together with their substratum from clean areas to areas under the influence of emissions. Mosses are exposed in polluted areas over a period of some weeks or months and next examined for heavy metals concentrations. This method is not recommended as, in addition to atmospheric pollution, the transplanted plants experience stress induced by other habitat factors like changes in light and humidity (Tyler, 1990; Ceburnis and Valiulis, 1999).

In moss bag method, samples of dried or fresh moss collected from clean areas are placed in nylon nets and exposed in a polluted area over different periods of time. Afterwards, the concentration of heavy metals is measured in the samples. The most often used moss species are *Sphagnum* spp. and more rarely *Hypnum cupressiforme* agg, *Hylocomium splendens* (Hedw.) B.S.G. or *Pleurozium schreberi* (Brid.) Mitt. The moss bag technique has been used most often in urban areas and industrial agglomerations where indigenous mosses are absent (Hynninen, 1986; Makinen, 1987; Yurukova and Ganeva, 1997; Ceburnis and Valiulis, 1999). The concentration of heavy metals in moss bags correlates with atmospheric heavy metals content. Tyler (1990) and Ceburnis and Valiulis (1999) recommend to use the moss bag technique in areas without indigenous moss flora. However, they also point to factors limiting the use of this method (type of bag, time of exposure). In Wisconsin USA moss bag technique was used to monitor heavy metals, sulfur and nitrogen using mesh bag containing *Sphagnum russowii* (Makholm and Mladenoff, 2005). Similar studies were carried out in Romania, Russia and Bulgaria using *Sphagnum girgensohnii* and 36 elements were investigated (Culicov *et al*., 2005).

The studies of impact of air pollutants on bryophytes have gained wider publicity in many continents of the world. The most important air pollutants associated with bryophytes include sulphur dioxide, lead (and other heavy metals), hydrocarbons, and nitrogen oxides. Bryophytes are sensitive to sulphur dioxide, which causes serious damage to their chloroplasts (Oyesiku, 2012). Ectohydric mosses have been used as biomonitors, in most cases, of trace-element atmospheric pollution. They possess many properties that make them suitable biomonitors for air pollutants (Onianwa, 2001; Zeichmeister *et al*., 2003) such as the following;

- They obtain nutrients from wet and dry deposition.
- They do not have real roots. So, they cannot take their nutrient from soil.

- Nutrient uptake from the atmosphere is promoted by their weakly developed cuticle.
- Large surface-to-weight ratio improves adsorption.
- Slow growth rate lets them accumulate pollutants over a larger time period.
- Undeveloped vascular bundles allow better adsorption than vascular plants.
- Minimal morphological changes during moss lifetime.
- Perenniality.
- Wide distribution.
- Ease of sampling.
- Possibility to determine concentrations in the annual growth segments.

Mosses are available in Nigeria and an earlier survey by Onianwa and Egunyomi (1983) has shown some local species to be suitable for biomonitoring atmospheric heavy metal pollution. Some studies were carried out in the country by various scientists (Onianwa *et al*., 1986; Onianwa and Ajayi, 1987; Kakulu, 1993; Fatoba and Oduekun, 2004; Bako *et al*., 2008; Adebiyi and Oyedeji, 2012; Ekpo *et al*., 2012) to monitor trace element air pollution using bryophytes as biomonitors. The species commonly used include *Calymperes palisotii, Erpodium coronatum, Brachymenium leptophylum, Polytrichum juniperinum, Barbula lambarenensis,* e .t. c.

2.5 Basis for the Use of Bryophytes as Biomonitors

The use of bryophytes in an increasing number of monitoring programs is based on a wide range of remarkable anatomical and physiological properties (Zechmeister *et al*., 2003). Air pollutants are deposited on mosses in three forms as aqueous solution, gaseous form or attached particles. Chakrabortty and Paratkar (2006) mentioned a number of different mechanisms pollutants accumulate in mosses: as layers of particles, entrapment on the surface of the cells, incorporation into the outer wall of cells through ion exchange process, metabolically controlled passage into the cells, e. t. c.

Mineral requirements of mosses are similar to those of higher plants. Mineral uptake by the cell is controlled by a semi permeable membrane. The protonema and the early gametophyte are attached to the substrate and significant stocks of nutrients may be accumulated from the surface at this stage (Zechmeister *et al.*, 2003). Later on, many pleurocarpus species leave the close contact to the substrate and it is generally assumed that the main source of minerals for these species are atmospheric sources (Tamm, 1953; Bates and Bakken, 1998), though some elements (e. g Ca, K, and P) seem to be derived further via the substrates (Bates, 1992; Wells and Boddy, 1995; Brown and Brumelis, 1996). Epiphytic mosses may be considered for common use as biomonitor organisms. This is largely based on their lack of roots when compared with higher plants. Thus they obtain their mineral supplies only from aerial sources and not from the substratum (Martin *et al.*, 1982).

Ion exchange is a fast physiological-chemical process that is affected by the number and type of free cat-ion exchange sites, the age of the cells, their reaction to desiccation, growing condition, temperature, precipitation, pH, composition of the pollutants and leaching (Tyler, 1990). In the ion exchange process, cat-ions and an-ions become attached to the functional organic groups in the cell wall primarily through chelation (Rao, 1984). Several investigations have shown that uptake efficiencies seem to follow the order Pb □ Co, Cr □ Cu, Cd, Mo, Ni, V, Sb □ Zn □ As (Chakrabortty and Paratkar, 2006; Zechmeister *et al*., 2003). Zechmeister *et al* (2003) also added that the total metal binding is determined by the number of available exchange sites and morphological structures of the bryophytes, which differs from species to species. There are no significant differences in the accumulation of a wide range of trace metals (e.g Pb, Cd, Cu, V) between mosses *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B. S. G. (Herpin *et al*., 1994; Halleraker *et al*., 1998).

2.6 Factors Modifying the Concentrations of Elements/Trace Metals in Moss

In addition to air pollutants that originate from anthropogenic sources, the concentrations in mosses are affected by many natural factors associated with morphological and physiological properties of the mosses as well as the site where the mosses are growing and their immediate environment (Chakrabortty and Paratkar, 2006). According to them, there are natural differences in chemical composition between individual species with different growths and conditions, and between separate parts of the individual moss. There are natural differences in chemical composition between individual species and even among populations of the same species.

Many years of surveys carried out by researchers in different countries in and beyond Europe have shown that the level of heavy metals in mosses can be influenced by factors other than air pollution such as climatic and edaphic factors, moss species, e. t. c (Zechmeister *et al*., 2003). Other factors affecting/modifying the heavy metal concentrations include nutrient status of the site, leaching from vegetation layers located above the mosses, precipitation intensity/frequency/duration, altitude due to changes in amount of precipitation/dust/biomass production, sampling and measuring method employed, age of the moss, mineral particles (mainly windblown dust from local soils), date of sampling (seasonal variation), type of moss sample (indigenous, transplanted, bag), e. t. c. According to Zechmeister *et al* (2003), moss methods are useful and often more useful than other biological methods in assessing atmospheric heavy metal pollution despite the variations, when properly applied.

2.7 Some Other Economic Importance of Bryophytes

Although bryophytes are among the oldest land plants, their usefulness is relatively unknown to most people. Bryophytes are important as model organisms in basic research since they are sound subjects for physiological and biochemical experiments. They have the advantage of being relatively simple plants, with a potentially rapid turnover of generations, and a dominant generation that is haploid; they are, therefore, particularly suited for genetic studies (Hallingback and Hodgetts, 2000). The teaching of botany can be greatly enhanced by using bryophytes; it is relatively easy to examine the leaf cells as they are transparent and usually only one cell thick. They are also good subjects for the study of reproduction as the antheridia and archegonia are often clearly visible and easy to dissect. Because examining their parts is relatively easy, they are ideal organisms for learning how to use a microscope. Bryophytes are used in pharmaceutical products, in horticulture, for household purposes, and are also ecologically important. The multifarious uses and applications of the bryophyte flora are being increasingly recognized around the world. Their potential in the biomapping of atmospheric precipitation is also enormous (Saxena and Harinder, 2004).

They play an important role in many ecosystems such as tundras, bogs and tropical rainforests (the extension of bogs in the boreal zone is much larger than that of the tropical rain forest, although this zone appears relatively small in certain map projections). In these ecosystems, bryophytes play an important role in water storage, nutrient uptake from rain and ecological interactions (habitat for animals) as stated by Frahm *et al*. (2003). Given to the bryophytes habitat information, bryology has a great relevance to agriculture in many ways. Simon (1975) demonstrated that bryophytes could be used as indicators of soil quality. The coarse textured mosses increase water storage capacity and fine textured ones provide air spaces in the soil (Ishikawa, 1974). Bryophyte screens not only help protect soil from wind and water erosion, but also provide suitable habitats for nitrogen fixing colonial endosymbiont Cyanobacteria. A typical example is *Nostoc*, which enters the hornwort (*Phaeoceros* spp.) and is established (Renzaglia and Vaughn, 2000).

Because of their sensitivity to water loss, bryophytes are good indicators of microclimate and altitudinal zonation of rain forests which makes them useful also in biodiversity research. Due to direct reaction to climatic factors and short life cycles and spore dispersal, bryophytes are also good and very fast indicators of climatic changes (Frahm *et al*., 2003). The occurrence of certain aquatic mosses can be used as an indicator of calcium and nutrient content in water. Some bryophytes grow only in a narrow and specific pH range and, therefore, their presence can be used as an indicator of soil pH. Bryophytes envelope the forest floor and tree trunks and aid in moisture conservation.

Mosses like *Atrichum* sp, *Pogonatum* sp, *Trematodon* sp, *Pohlia* sp, *Nardia* sp and *Blasia* sp play a role as inhibitors of soil erosion due to their trample-resistant structure and their regenerative ability. Acrocarpous mosses like *Rhodobryum* and *Dicranum* prevent soil erosion of the slopes of hills because the netted and webbed protonemata cover the exposed substrata. In pastures of Nova Scotia in Canada, it has been seen that white spruce germinates most prolifically in carpets of *Polytrichum*. Many mosses, especially *Hypnum imponens*, provide excellent seeding beds for a variety of coniferous tree species. This is likely to be due to the role of bryophytes in providing moisture, appropriate temperature, and also organic matter and minerals after the death of bryophytes. Thus, bryophytes play an important role in the maintenance and replenishment of forest cover (Saxena and Harinder, 2004).

The phytochemical study of bryophytes for pharmaceutical “lead” compounds has been neglected because of their tiny nature which makes it difficult to identify and to collect large quantity of pure sample for producing drugs (Asakawa, 2001). However, recent studies of mosses and liverworts *in vitro* have shown that they synthesize distinct antibiotically active substances (Dulger *et al*., 2005; Ilhan *et al*., 2006; Ojo *et al*., 2007). Moreover, various secondary metabolites present in bryophytes are responsible for bioactive metabolites. Such metabolites are effective as antitumor, antibiotics, anti-fungal, anti-feedants, and repellents (Huneck, 1983; Spjut, 1986; Brinkmeier *et al*., 1999; Asakawa, 2001; Harinantenania *et al*., 2006; Sabovljevic *et al*., 2006). Asakawa (2001), reported on complex phenolic compound bibenzyls among the liverworts. The only metabolite not found in mosses is alkaloid. According to Mann (1978), primitive plants contain tannins whereas the modern plants contained alkaloids. This translates to mean that bryophytes are primitive in nature. The Chinese and the native Americans have used various moss species like *Philonotis, Bryum, Mnium,* crushed into a kind of paste and applied as a poultice. In India, the burned ash of mosses mixed with fat and honey is used as an ointment for cuts, burns and wounds in the Himalayan region (Saxena and Harinder, 2004).

Chinese traditional medicine names 40 kinds of bryophytes that have been used to treat illnesses of the cardio-vascular system, tonsillitis, bronchitis, tympanitis, cystitis, as well as skin diseases and burns. It has been shown that an extract of *Rhodobryum giganteum* can increase aorta blood transit by up to 30% in animals. Transgenic *Physcomitrella* are now being used to produce 'blood-clotting factor IX', for the treatment of 'haemophilia' B (Saxena and Harinder, 2004). Chemical analysis has revealed that most bryophytes, including *Sphagnum*, have antibiotic properties (Banerjee, 1974). Extracts of many species of mosses and liverworts contain phenolic compounds that inhibit growth of pathogenic fungi and bacteria. Dried *Sphagnum* is, therefore, an excellent surgical dressing because of its absorptive qualities (absorbing more liquid than cotton pads [Richardson, 1981]), and its ability to prevent infection. Because of these properties, it was used extensively during World War I (Hallingback and Hodgetts, 2000).

Liverworts and mosses have long been tried and used as a fuel in developed countries like Finland, Sweden, Ireland, West Germany, Poland and Soviet Union. Peat is suitable for production of low and intermediate BTU gas as well as hydrogen, ethylene, natural gas, methanol and Fisher Tropsch gasoline. Peat has rapid regeneration, can be easily harvested, has low sulfur content, and its heating value is superior to that of wood (Saxena and Harinder, 2004). *Sphagnum* moss has been used as an effective filtering and absorption agent for the treatment of waste water and effluents from factories with acid and toxic discharges containing heavy metals, organic substances such as oils, detergents, and dyes (Poots *et al*., 1976), and micro-organisms (Rozmey and Kwiatkowski, 1976).

Sphagnum is often the most important plant in bogs and in peat formation (Hallingback and Hodgetts, 2000). Peat is the accumulated and compressed remains of vascular and non-vascular plants (mainly bryophytes, particularly *Sphagnum*). Peat lands are recognized as carbon sinks and it is, therefore, important that they remain undisturbed. Human activities, including drainage, fertilization, and peat land cultivation, can increase the amount of carbon dioxide released from peat, owing to increases in microbiological activity. These disturbed peat lands then become sources rather than sinks for carbon in the global ecosystem (Francez and Vasander, 1995). Bryophyte communities are critical to the survival of a tremendous diversity of organisms, including insects, millipedes, and earthworms. Numerous arthropods, such as acarinae and collembola, and tardigrades, are dependent on mosses and liverworts as habitat, or as a food source. The nutrient-rich, spore-producing capsules are particularly palatable to some insects, and molluscs such as slugs. Bryophytes are also a food source for birds and mammals in cold environments, and are eaten by reindeer, geese, ducks, sheep, musk-ox, lemmings, and other rodents (Longton, 1992).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Description of Study Areas

The study areas as shown in Figure 3.1 are Kano, Zaria, Ringim and Birnin Gwari all in the North-Western region of Nigeria, and a number of criteria were considered in their choice.

Kano is located at 12° 00′ 00.00″ N and 8° 31′ 00.00″ E. It has a substantial amount of industries, with commercial activities, heavy traffic density, densely populated and highly urbanized.

Zaria located at 11° 04′ 54.91″ N and 7° 42′ 57.44″ E is less industrialized, but with heavy traffic density, densely populated and highly urbanized.

Ringim (12° 08′ 50.14″ N and 9° 10′ 09.27″ E) is more of a rural setting and not industrialized, with moderate traffic density, moderately populated and urbanized.

The Kamuku National Park in Birnin Gwari on the other hand is relatively “pollution free” with very little human activities and is located at 10° 40′ 00.00″ N and 6° 32′ 00.00″ E.

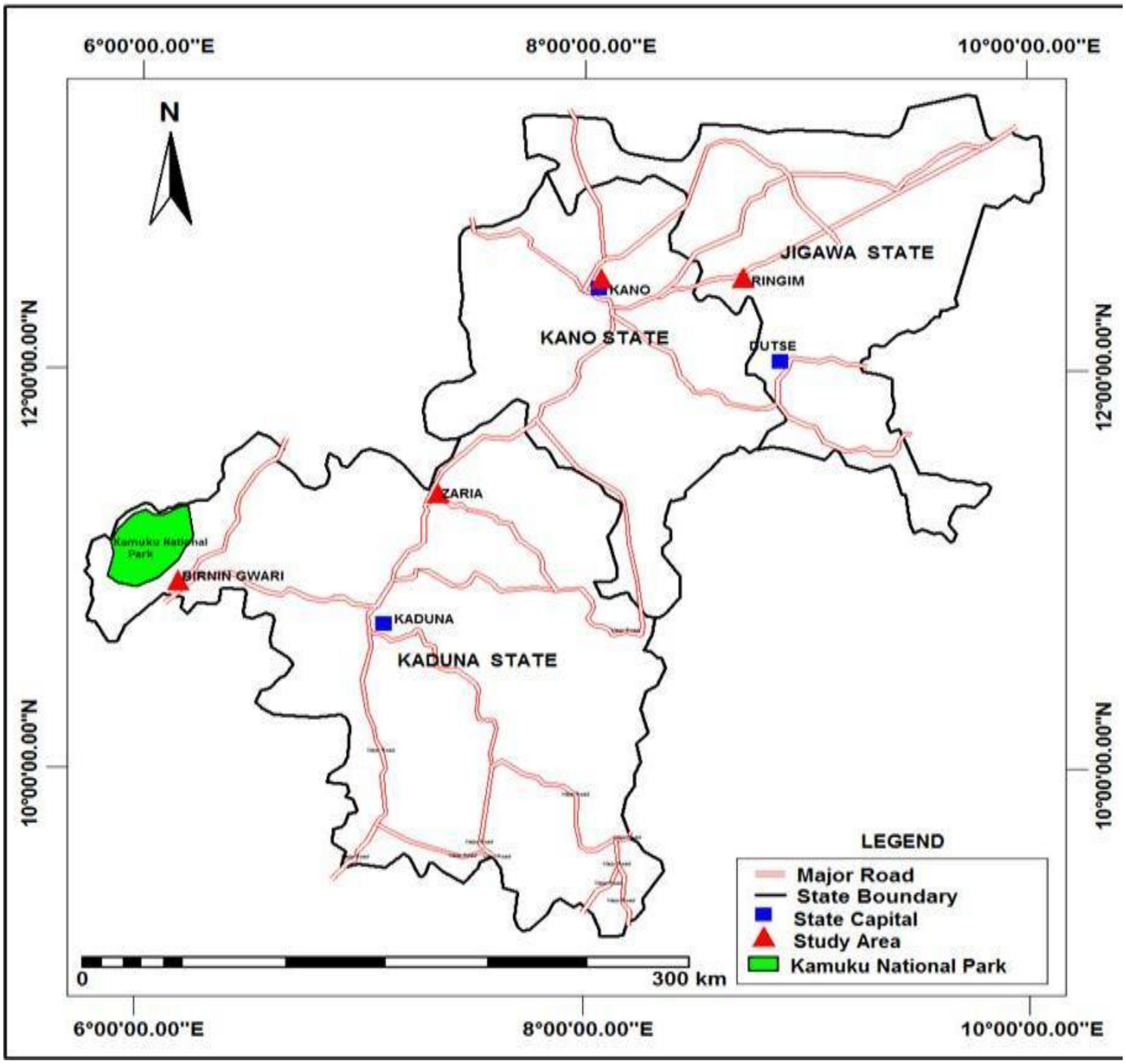

Fig. 3.1. Location of the Study Areas

Source: Adapted and Modified from Administrative Map of Nigeria (2013).

3.2 Location of Study Sites

Sites selected in each location/area were located at least 300 meters from main roads, densely populated areas and other direct source of pollutants. This was to assist in the collection of samples in the areas suspected to be of low deposition of all the pollutants, and also to avoid collection of samples from areas of pollution sources. Wherever possible, the site selection for this study took into consideration the reproducibility of results and other environmental factors such as accessibility, availability of open spaces, and of course areas with minimal local influence from traffic as well as other anthropogenic activities.

3.3 Sampling of Moss Species

Moss sampling was carried out during the dry and wet seasons, 2013. Specifically samples were collected during three months of dry season and three months of wet season. For each month, moss samples were collected from at least six sites in each location selected. The moss species were collected from 2 to 2.5 m high from the ground on trees, uncompleted buildings, plastered and un-plastered perimeter fences (walls), gutter walls, rock surfaces, forest floor/soil/sand and such structures within the sampling area. The samples were placed in small brown envelopes, labeled accordingly with field notes and then taken to the laboratory for further analysis. Unidentified samples were taken to the Herbarium of the Department of Botany, University of Ibadan, Nigeria, for proper identification.

3.4 Distribution/Occurrence of Bryoflora on Substrates

The distribution/occurrence of the plants on the various substrates was calculated using the formulas adopted by Adebiyi and Oyeyemi (2013), as follows:

% Occurrence of moss species in all locations

$$= \frac{\text{Total number of each moss species found in all locations}}{\text{Total number of the moss species found in all locations}} \times 100$$

% occurrence of moss species on all the substrates

$$= \frac{\text{Total number of each moss species found on each substrate}}{\text{Total number of the moss species found on all substrates}} \times 100$$

% occurrence of moss species on each substrate

$$= \frac{\text{Total number of each moss species found on each substrate}}{\text{Total number of moss samples collected from each substrate}} \times 100$$

3.5 Preparation of Samples

The method of Shakya *et al.* (2001) and Ekpo *et al.* (2012) was used. Foreign materials adhering to the surface of the moss samples were removed carefully under dry conditions. Only the green and greenish brown parts of the plants were left for analysis since they were generally intended to represent a period of about 3-5 years. Their metal content is considered to reflect the atmospheric deposition during that period (Ruhling and Steinnes, 1998). The samples were first thoroughly washed with double-distilled water 3 times. They were then dried at 60℃ in an oven for 48 hours after which they were ground in a porcelain mortar to obtain fine particles and stored in polythene bags ready for analyses. The representative samples of each moss species were used for analyses in duplicates.

3.6 Determination of Heavy Metals

About 0.5 g of each representative sample was transferred into an open quartz tube for digestion. Concentrated HNO_3 and HCl were added to each tube in the ratio 3:1 and the mixture heated for about 2 hours. The solution was allowed to cool and then filtered through Whatman filter paper and the volume of the filtrate was diluted to 100ml with deionized water (Sawidis *et al.*, 1993; Chettri, 1997). The metal contents in the filtrates (Co, Ni, Cu, Cd, Pb, Cr, and Zn) were determined using Fast Sequential Atomic Absorption Spectrophotometer (Varian AAS 240FS).

3.7 Determination of NO_3 and NO_2 by Steam Distillation

For NO_3^- analysis, 0.2 g of each sample was weighed into distillating flask. About 25 g of Devarda's alloy and 10 ml of distilled water were added. The flask was attached to distillation apparatus. Then 10 ml of 2 % boric acid was measured into 50 ml conical flask and 2-3 drops of mixed indicator (methyl red and methyl blue) was added into the boric acid. The boric acid in the flask was attached to a condenser and the nitrate (NO_3) collected into the boric acid to about 50 ml volume. After collecting the distillate, it was titrated with 0.025 N H_2SO_4 to get the titer reading. The percentage nitrate was evaluated using the formula

% nitrate = $\frac{T*NA*100}{W}$

Where T = Titer volume

NA = Normality of acid

W = Weight

For the NO_2 analysis, (NO_2 + NO_3) was determined using above procedure, but in this case 0.5 g of MgO and 0.5 g of Devarda's alloy were added into the sample, followed by adding 10mls of distilled water. The distillate was collected using 2 % boric acid as above. The NO_2 was obtained by subtracting the initial NO_3 value from this final result obtained (Iwuofor *et al*., 1990; Agbenin, 1995; Horneck and Miller, 1998).

3.8 Determination of SO_2

About 0.2 g of each sample was weighed into a dry digestion tube and 5 ml of 2:1 nitric-perchloric acid was added. A small glass funnel to act as a reflux condenser was inserted and left over night. The tubes were placed in a heating block and digested for one hour at 150℃. Time when all tubes have reached the dense white fume stage was noted. This stage is not reached until essentially all the nitric acid has been driven off. The digestion was continued for 2 hours, the tubes removed from digestion block, cooled to about 100℃ and 1ml 1:1 HCl added to dispel the last traces of oxides of nitrogen. It was heated to white fuming stage again, removed from digestion block and cooled to handle. Water was added to 50ml volume, mixed thoroughly and left until silica has settled.

Sulfur in the digest was then determined by turbidimetry. Here, 10 ml of the sample aliquot was pipetted into a 25 ml volumetric flask, adding distilled water to bring the volume to approximately 20 ml. 1 ml of Gelatin ($BaCl_2$) reagent was added making up the volume with distilled water. The content was mixed thoroughly allowing standing for 30 minutes. The % T and O.D were determined at 420 m*u* within 30 to 60 minutes on a

Bosch and Lomb Spectronic – 70 electrocolorimeter. The content was shaken in the flask before pouring into the photo-test tube. Set of standard Sulphur solutions were prepared containing 0, 25, 50, 75, 100, 125 μg SO_4-S per 25 ml from the working standard solution (Tabatai, 1974; Miller, 1998).

3.9 Statistical Analyses

- Mean levels of the pollutants at the period of sampling for all locations and for all moss species were calculated.
- ANOVA was used to test for differences in individual concentrations in each species found and in the different locations.
- Duncan’s Multiple Range Test (DMRT) was used to separate the means in case they are significantly different.
- T-test was used to determine seasonal variability of mean levels of pollutants in moss species.

CHAPTER FOUR

4.0 RESULTS

4.1 Distribution/Occurence of Bryoflora

A total of about 144 samples were collected throughout, representing eight (8) different species of moss plants which are *Hyophila crenulata* C. Mull. ex Dus; *Barbula lambarenensis* C. Mull; *Bryum coronatum* Schwaegr; *Splachnobryum subjulaceum* Card.; *Brachymenium leptophyllum*; *Erpodium pobeguinii* Par & Broth; *Fabronia pilifera* Hornsch and *Fissidens grandifolius* Broth & P. Varde.

Out of the eight (8) species recorded, *Hyophila crenulata* was having the highest percentage of occurrence, followed by *Erpodium pobeguinii, Barbula lambarenensis* and *Bryum coronatum* while the least percentage of occurrences was recorded in *Fissidens grandifolius*. *Hyophila crenulata* and *Bryum coronatum* were found across all the four locations. All the eight species were seen during the wet season, but only *Hyophila crenulata, Barbula lambarenensis* and *Erpodium pobeguinii* were observed during both the dry and wet seasons. The percentage occurrence of all the moss species in all locations were presented in Table 4.1. More species were found in Zaria and Kamuku especially in the wet season and the least found in Kano and Ringim.

Table 4.1 Percentage occurrence of moss species in all locations across seasons in North West Nigeria

Moss species	**location(s)**	**season(s)**	**frequency**	**%occurrence**
Hyophila crenulata	Zr, Km, Kn, Rn	D, W	84	58.33
Barbula lambarenensis	Zr	D, W	12	8.33
Bryum coronatum	Zr, Km, Kn, Rn	W	6	4.17
Splachnobryum subjulaceum	Zr	W	4	2.78
Brachymenium leptophyllum	Zr	W	4	2.78
Erpodium pobeguinii	Zr, Km	D, W	28	19.44
Fabronia pilifera	Km	W	4	2.78
Fissidens grandifolius	Km	W	2	1.39
Total			144	100

Key: Zr=Zaria, Km=Kamuku, Kn=Kano, Rn=Ringim.

D=Dry season, W=Wet season.

Moss species were distributed among different substrates including bark of trees, sandcrete materials (plastered and un-plastered walls, blocks, rock surface) and sand/soil/forest floor. *Hyophila crenulata* were found growing on all substrates with majority on sandcrete materials; *Barbula lambarenensis, Bryum coronatum* and *Splachnobryum subjulaceum* were found only on sandcrete materials (100 % occurrence each); *Erpodium pobeguinii* mostly on bark of trees with some on sandcrete materials; *Fabronia pilifera* completely on bark of trees (100 % occurrence) and *Fissidens grandifolius* found growing only on sand/soil/forest floor (100 %). Sandcrete materials recorded the highest number of species, followed by bark of trees and then soil/sand with very few species. The occurrences were shown in Table 4.2 and the percentage occurrences on all substrates and on each substrate were presented in Tables 4.3 and 4.4 respectively.

Table 4.2 Occurrence of moss species on different substrates across seasons in North-West Nigeria

Moss species	**Substrates**		
	Bark of trees	**Sandcrete Materials**	**Soil/Sand/Forest floor**
Hyophila crenulata	4	76	4
Barbula lambarenensis	0	12	0
Bryum coronatum	0	6	0
Splachnobryum subjulaceum	0	4	0
Brachymenium leptophyllum	4	0	0
Erpodium pobeguinii	24	4	0
Fabronia pilifera	4	0	0
Fissidens grandifolius	0	0	2
Total	36	102	6

Table 4.3 Percentage occurrence of moss species on all substrates

Moss species		**Substrates**		**total%**
	Bark of trees	**Sandcrete Materials**	**Soil/Sand**	
Hyophila crenulata	4.76	90.48	4.76	100
Barbula lambarenensis	0	100	0	100
Bryum coronatum	0	100	0	100
Splachnobryum subjulaceum	0	100	0	100
Brachymenium leptophyllum	100	0	0	100
Erpodium pobeguinii	85.71	14.29	0	100
Fabronia pilifera	100	0	0	100
Fissidens grandifolius	0	0	100	100

Table 4.4 Percentage occurrence of moss species on each substrate

Moss species	**Substrates**		
	Bark of trees	**Sandcrete Materials**	**Soil/Sand**
Hyophila crenulata	11.11	74.51	66.67
Barbula lambarenensis	0	11.76	0
Bryum coronatum	0	5.88	0
Splachnobryum subjulaceum	0	3.92	0
Brachymenium leptophyllum	11.11	0	0
Erpodium pobeguinii	66.67	3.92	0
Fabronia pilifera	11.11	0	0
Fissidens grandifolius	0	0	33.33
Total %	100	100	100

4.2 Mean Levels of Heavy Metal Concentrations in Different Locations

A summary of the mean levels of seven (7) heavy metal concentrations in all locations was given in Table 4.5. It was found that the concentrations of all the heavy metals vary significantly both within and among the study locations ($p < 0.05$). Species found across all locations were used for comparison among the various locations. Kano was having the highest mean concentrations of 4 of the 7 heavy metals analyzed which are Cu, Cd, Cr and Pb; Zaria having the highest concentration of Zn while Kamuku the highest of Co. From the concentrations, it is clear that Kano is more contaminated with almost all the heavy metals investigated, followed by Zaria and then less contamination in Kamuku and Ringim. In general, the mean concentration of Zn was highest and that of Cd was the lowest in all locations. The total average abundance of each metal and in each location was in the following order: Zn > Cr, Cu, Pb > Ni > Co > Cd. A comparison of each individual heavy metal in all the 4 locations was given in Figure 4.1.

Table 4.5 Mean levels of heavy metal concentrations (ppm) in different locations

	Zaria	**Kamuku**	**Kano**	**Ringim**
Ni	10.38±1.17^{a}	10.45±1.63^{a}	10.25±0.79^{a}	8.29±0.62^{b}
Cu	20.09±4.8^{c}	35.38±24.41^{b}	68.65±5.3^{a}	14.77±1.23^{d}
Cd	0.42±0.08^{b}	0.10±0.02^{c}	2.56±1.26^{a}	0.25±0.01^{bc}
Pb	22.70±1.29^{b}	14.59±3.69^{c}	67.39±50.06^{a}	14.44±1.84^{c}
Cr	30.59±5.55^{b}	20.2±7.99^{c}	96.75±20.28^{a}	12.99±6.92^{d}
Zn	250.47±107.34^{a}	38.41±3.75^{d}	144.38±8.5^{b}	66±11.17^{c}
Co	1.78±1.29^{c}	8.91±2.33^{a}	4.87±0.83^{b}	1.16±0.14^{c}

Values with same superscripts along the rows are not significantly different ($P \geq 0.05$)

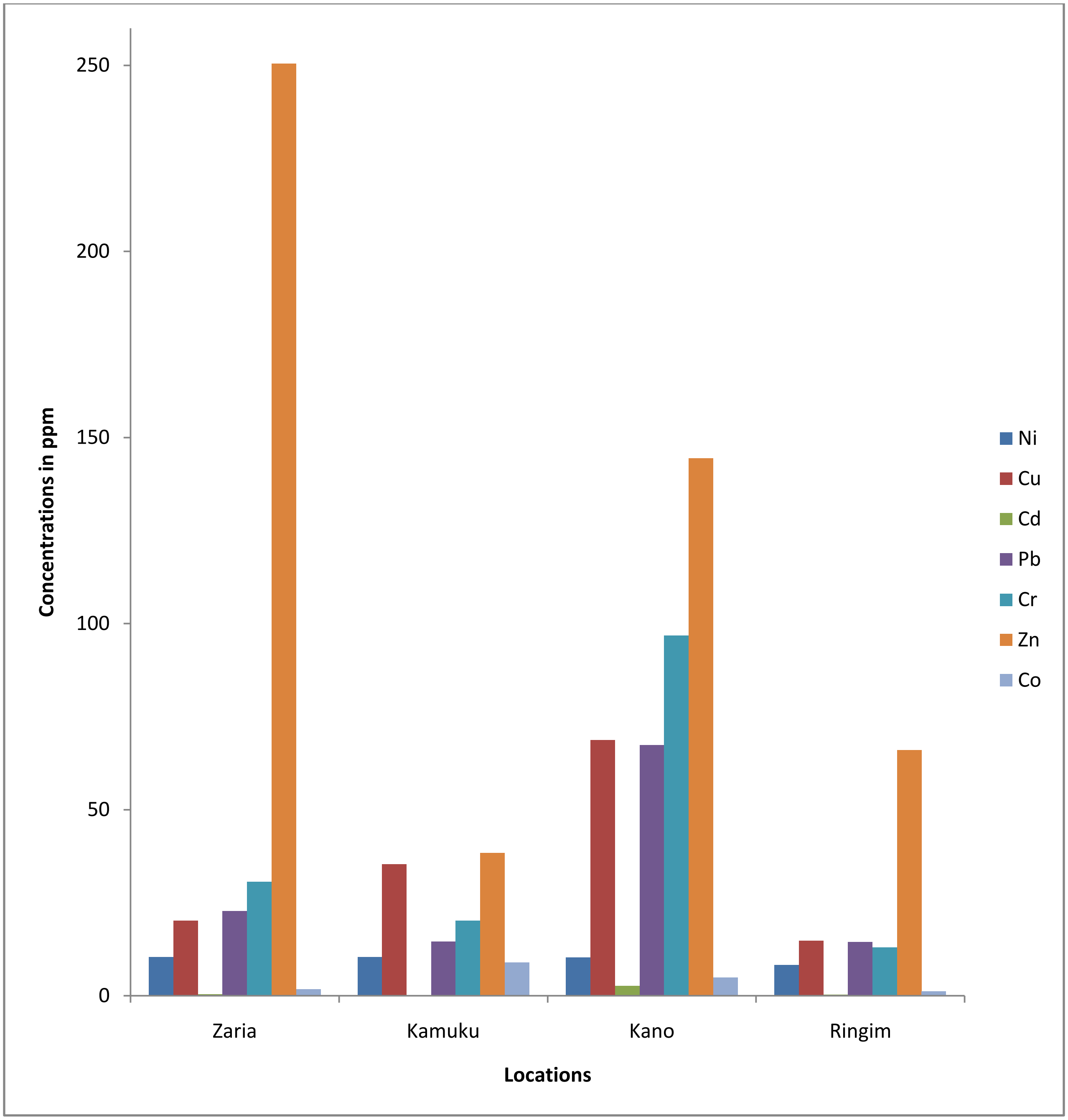

Fig. 4.1 Comparison of metal concentrations in different locations

4.3 Seasonal Variation in the Levels of Heavy Metal Concentrations in Different Locations

Table 4.6 shows the seasonal variation in the mean levels of metal concentrations. Ni, Cu, Cd, Cr and Co showed relatively higher accumulation values in the wet season than the dry season, while Pb and Zn had higher values in the dry season but statistically not significant in all cases with $p > 0.05$ as indicated in the table.

Table 4.6 Seasonal variation in the mean level of metal concentrations (ppm)

	Dry	Wet	P value	Remark
Ni	9.09±2.45	12.39±2.00	0.32	NS
Cu	22.69±3.50	38.85±4.56	0.41	NS
Cd	1.02±0.20	1.08±0.33	0.93	NS
Pb	39.09±7.62	34.49±3.43	0.84	NS
Cr	28.08±6.55	36.59±3.22	0.72	NS
Zn	112.89±10.12	91.62±4.66	0.72	NS
Co	3.68±1.20	6.58±1.56	0.33	NS

NS=Not significant ($p \geq 0.05$)

4.4 Mean Levels of Heavy Metal Concentrations in Different Moss Species

The mean levels of the heavy metal concentrations in different moss species were summarized in Table 4.7. There was a significant difference in the uptake and accumulation of all the metals by different moss species ($p < 0.05$), and most of the species in turn showed preference for certain heavy metals. A comparison of the elemental concentrations in the moss species was carried out to see their differences in accumulation and uptake efficiency of the heavy metals.

Bryum coronatum was found to have the highest concentrations of four (4) of the seven (7) analyzed metals which are Cu, Pb, Cr, and Zn; *Fissidens grandifolius* having the highest concentrations of Ni and Co while *Fabronia pilifera* with the highest concentration of Cd. *Hyophila crenulata* had the second highest concentrations of Cu, Pb, Cr and Co. There was no accumulation of Cr and Co recorded in *Fabronia pilifera*. Zn concentration was highest than other heavy metals in all the species while Cd had the least concentration in all. In general, the trend of accumulation in the moss species was in the following order: Zn > Pb, Cr, Cu > Ni > Co > Cd. A comparison of metal concentrations in the different Moss species was given in Figure 4.2. Also, a comparison of individual metal concentrations of different moss plants at different locations were given in Figures 4.3-4.9 to give an overview of best moss species for each heavy metal in each location.

Table 4.7 Mean levels of heavy metal concentrations (ppm) in different Moss species

	Ni	**Cu**	**Cd**	**Pb**	**Cr**	**Zn**	**Co**
H. crenulata	9.59±0.56^{bc}	36.13±10.59^{b}	0.9±0.51^{b}	30.97±14.49^{b}	40.73±15.40^{b}	122.19±42.89^{c}	4.34±1.46^{b}
B. lambarenensis	10.36±1.18^{b}	17.2±7.69^{c}	0.17±0.10^{d}	30.14±8.77^{b}	25.89±10.26^{c}	95.83±47.31^{cd}	3.56±0.49^{b}
B. coronatum	10.86±0.35^{b}	57.95±38.03^{a}	1.52±1.32^{a}	99.88±32.19^{a}	52.88±46.94^{a}	559.44±389.03^{a}	4.43±2.21^{b}
S. subjulaceum	9.91±0.79^{bc}	16.4±0.84^{c}	0.29±0.25^{d}	26.3±5.28^{c}	27.3±2.07^{c}	119.94±56.79^{c}	2.33±2.01^{c}
B. leptophyllum	11.68±2.30^{b}	16.54±1.15^{c}	0.8±0.21^{b}	24.56±3.35^{c}	11.71±1.52^{e}	71.06±5.16^{d}	2.12±0.21^{c}
E. pobeguinii	8.7±1.04^{c}	17.19±0.54^{c}	0.85±0.20^{b}	28.45±3.19^{b}	20.39±6.04^{d}	64.2±11.43^{d}	3.72±0.78^{b}
F. pilifera	7.56±0.76^{c}	13.61±1.31^{d}	1.58±1.12^{a}	25.41±3.42^{c}	0±0.00	163.13±20.55^{b}	0±0.00
F. grandifolius	31.86±3.13^{a}	7.47±0.16^{e}	0.54±0.07^{c}	12.5±2.11^{d}	4.15±0.55^{f}	16.86±3.02^{e}	18.24±3.40^{a}

Values with same superscripts along the columns are not significantly different ($P \geq 0.05$)

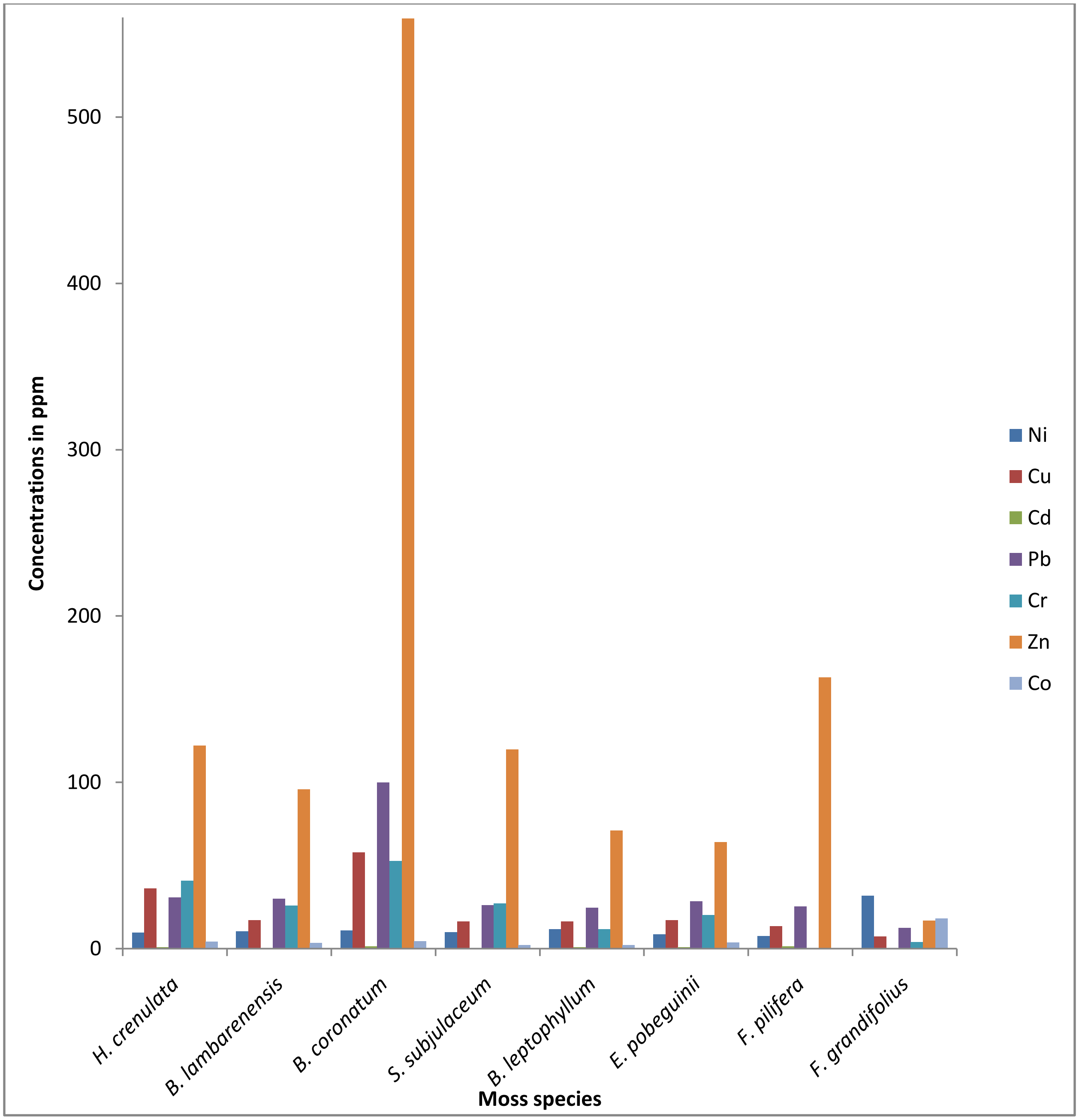

Fig. 4.2 Comparison of metal concentrations in different Moss species

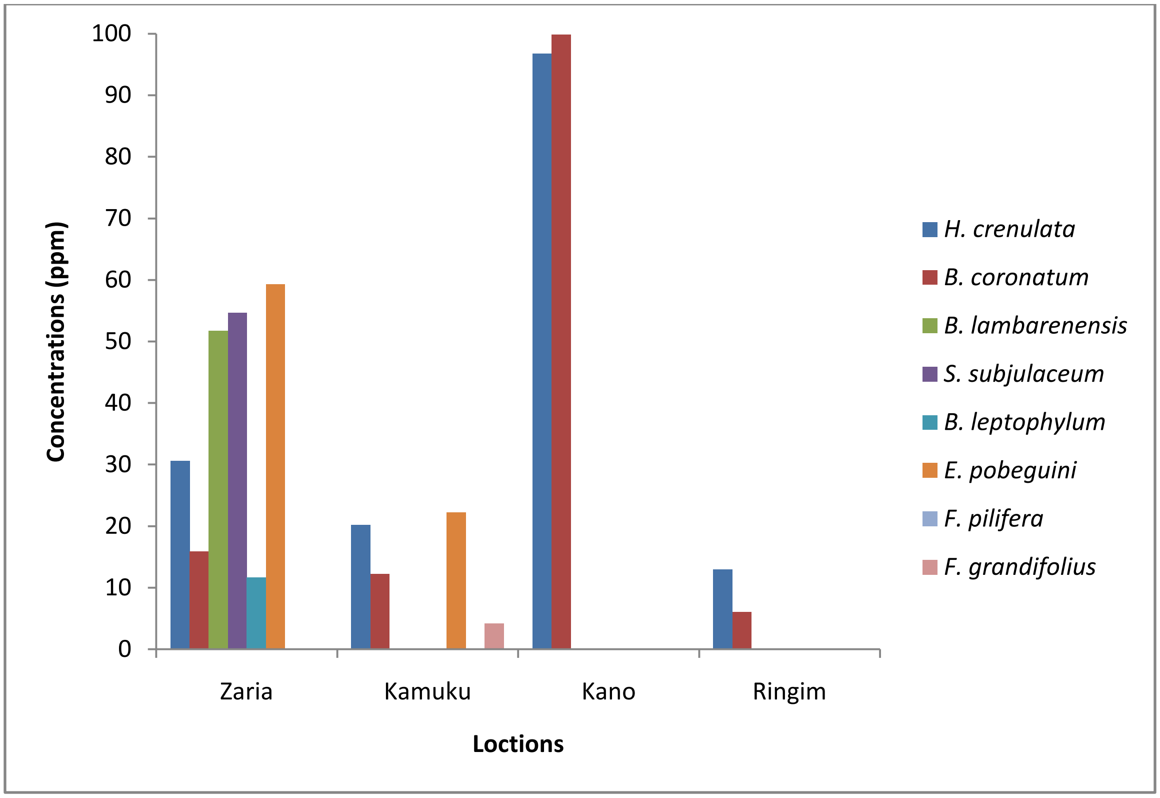

Fig. 4.3 Accumulation of Cr by different moss species at different locations

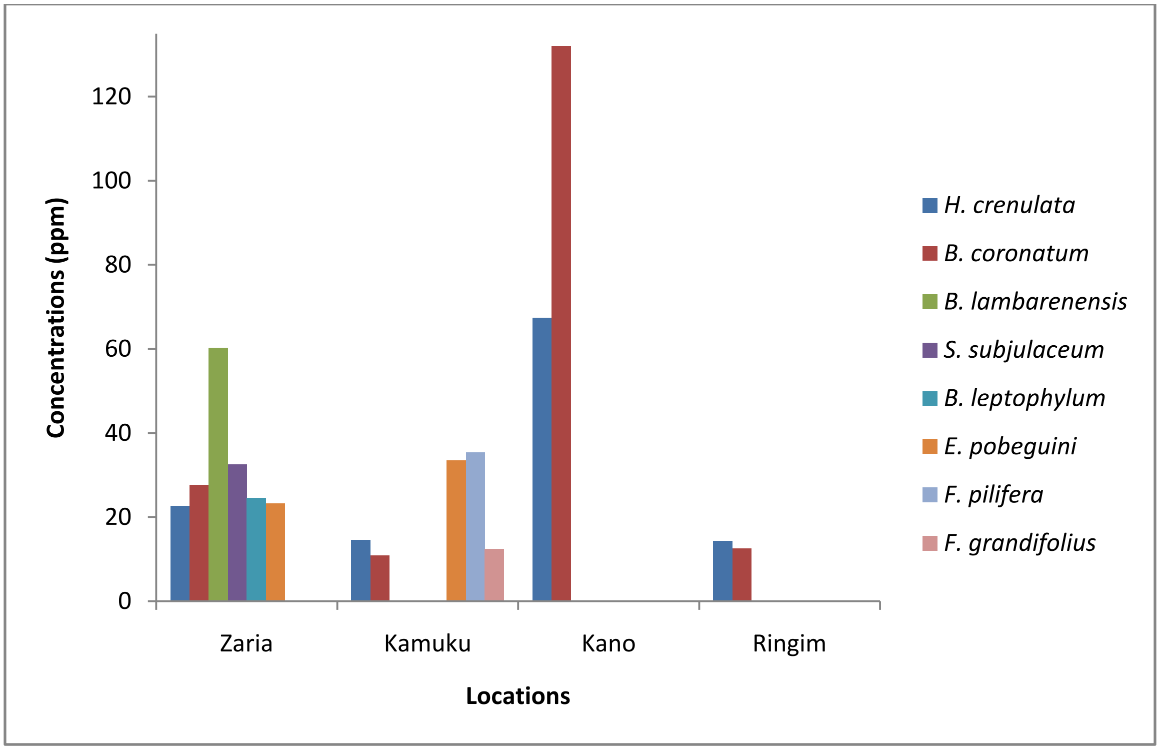

Fig. 4.4 Accumulation of Pb by different moss species at different locations

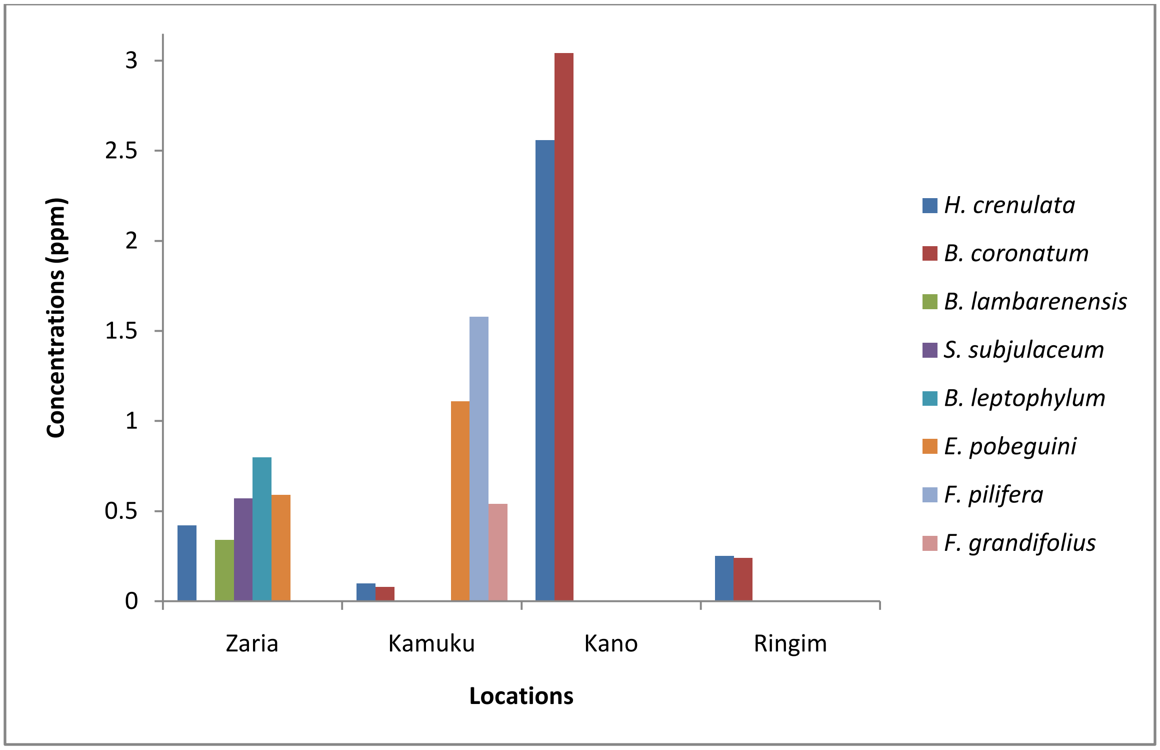

Fig. 4.5 Accumulation of Cd by different moss species at different locations

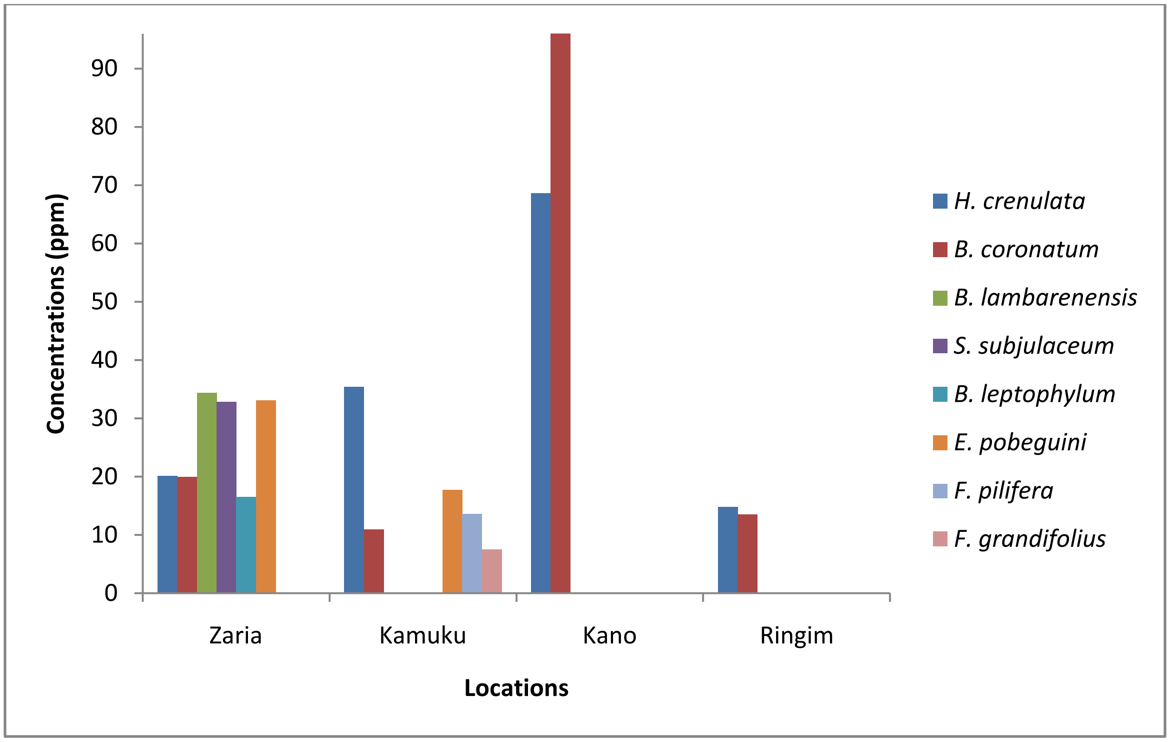

Fig. 4.6 Accumulation of Cu by different moss species at different locations

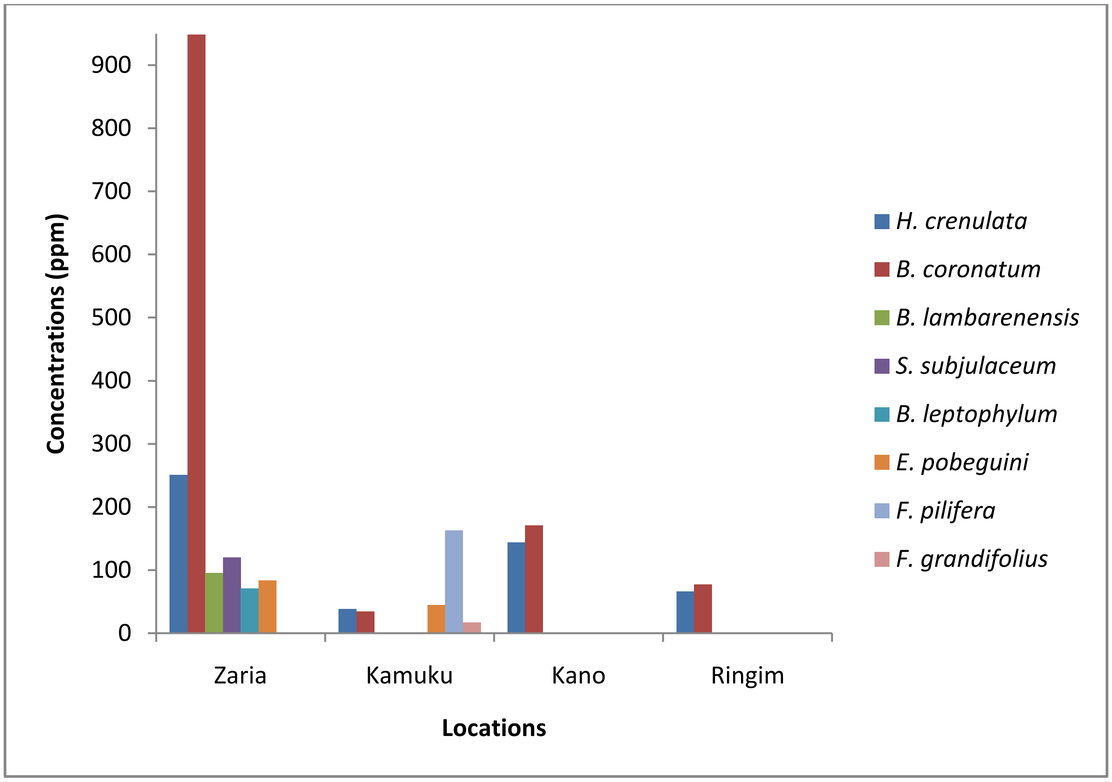

Fig. 4.7 Accumulation of Zn by different moss species at different locations

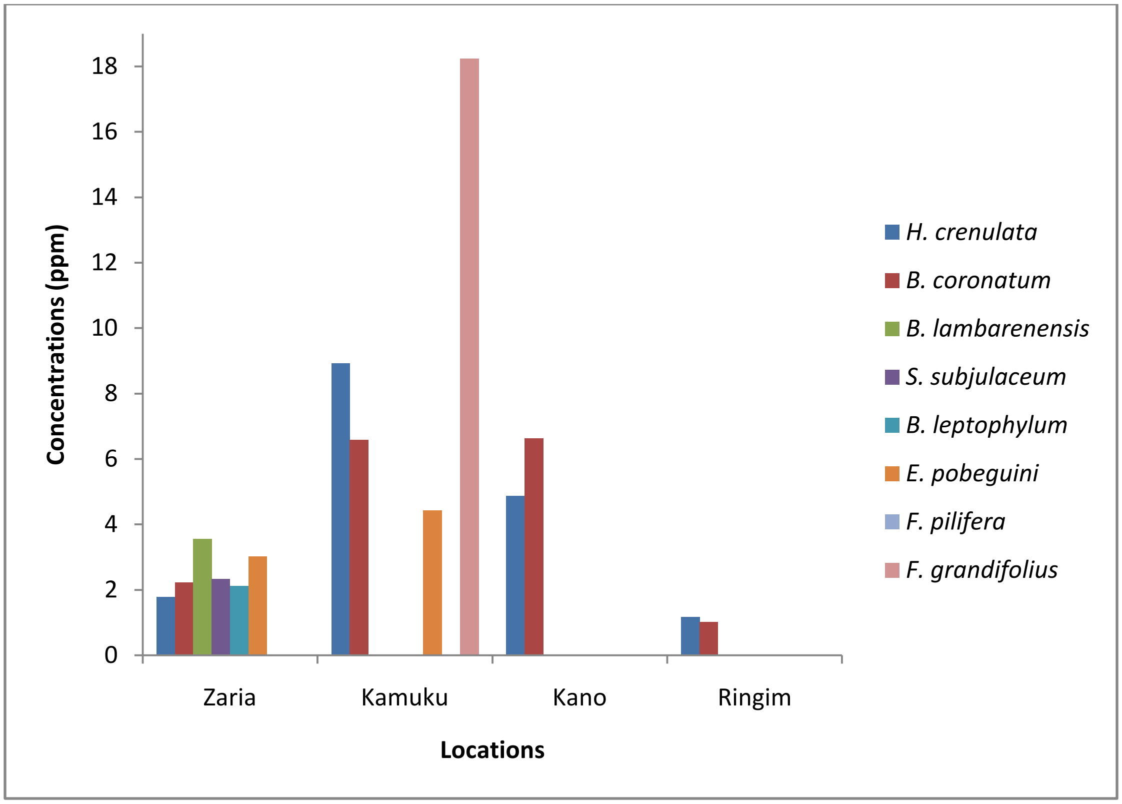

Fig. 4.8 Accumulation of Co by different moss species at different locations

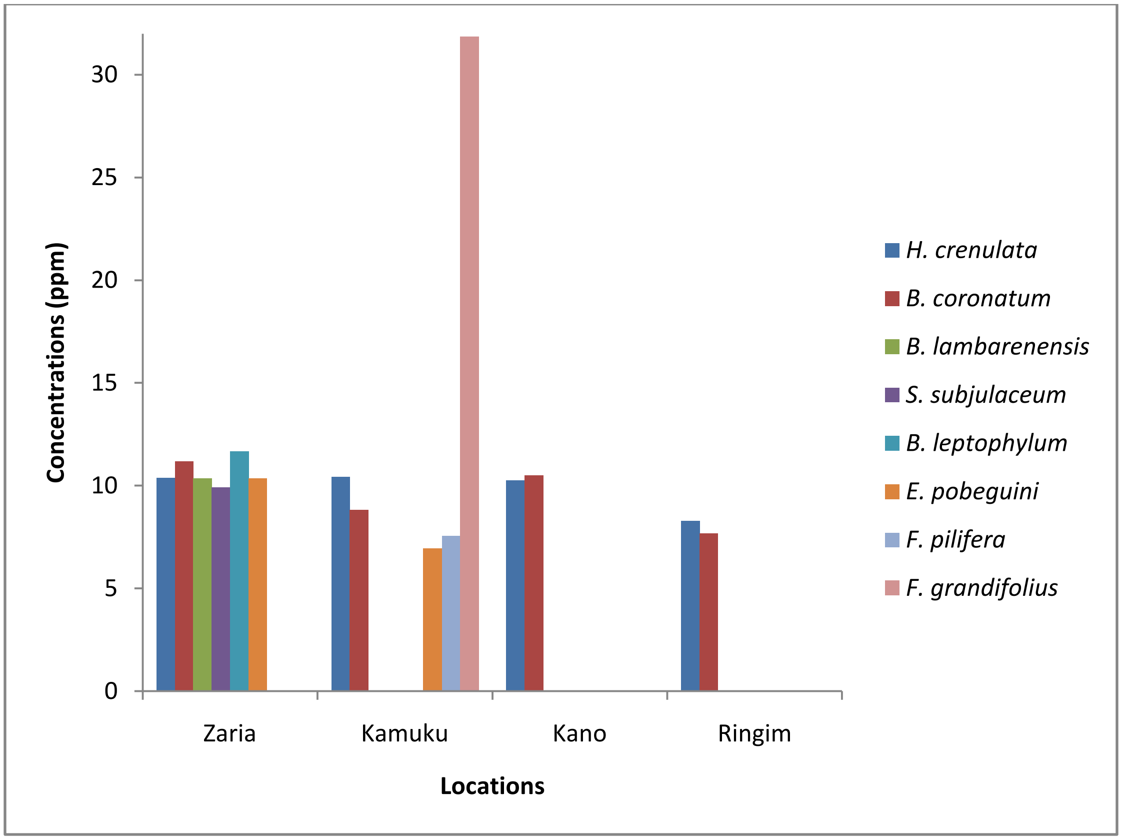

Fig. 4.9 Accumulation of Ni by different moss species at different locations

4.5 Mean Levels of SO_2, NO_2 and NO_3 Concentrations in Different Locations

Table 4.8 shows the summary of the mean levels of SO_2, NO_2 and NO_3 concentrations in the study locations. The concentrations of the SO_2, NO_2 as well as that of NO_3 vary significantly both within and among the study locations ($p < 0.05$). Species found across all locations were used for comparison among the various locations. The highest concentration of the SO_2 was observed in Zaria and the least in Kamuku, while highest concentrations of both NO_2 and NO_3 were observed in Kamuku and least in Zaria. In all the locations, the concentration of NO_3 is highest, followed by that of NO_2 with that of SO_2 as the least, as shown in Figure 4.3.

Table 4.8 Mean levels of SO2, NO2 and NO3 concentrations (ppm) in different locations

	SO_2	NO_2	NO_3
Zaria	513.2 ± 30.20^{a}	1170 ± 130^{c}	1575 ± 175^{c}
Kamuku	150.94 ± 30.18^{c}	3250 ± 650^{a}	4375 ± 875^{a}
Kano	362.26 ± 0.20^{b}	2350 ± 1550^{b}	3150 ± 2100^{b}
Ringim	482.5 ± 0.50^{ab}	1430 ± 130^{c}	1925 ± 175^{c}

Values with same superscripts along the columns are not significantly different ($P \geq 0.05$)

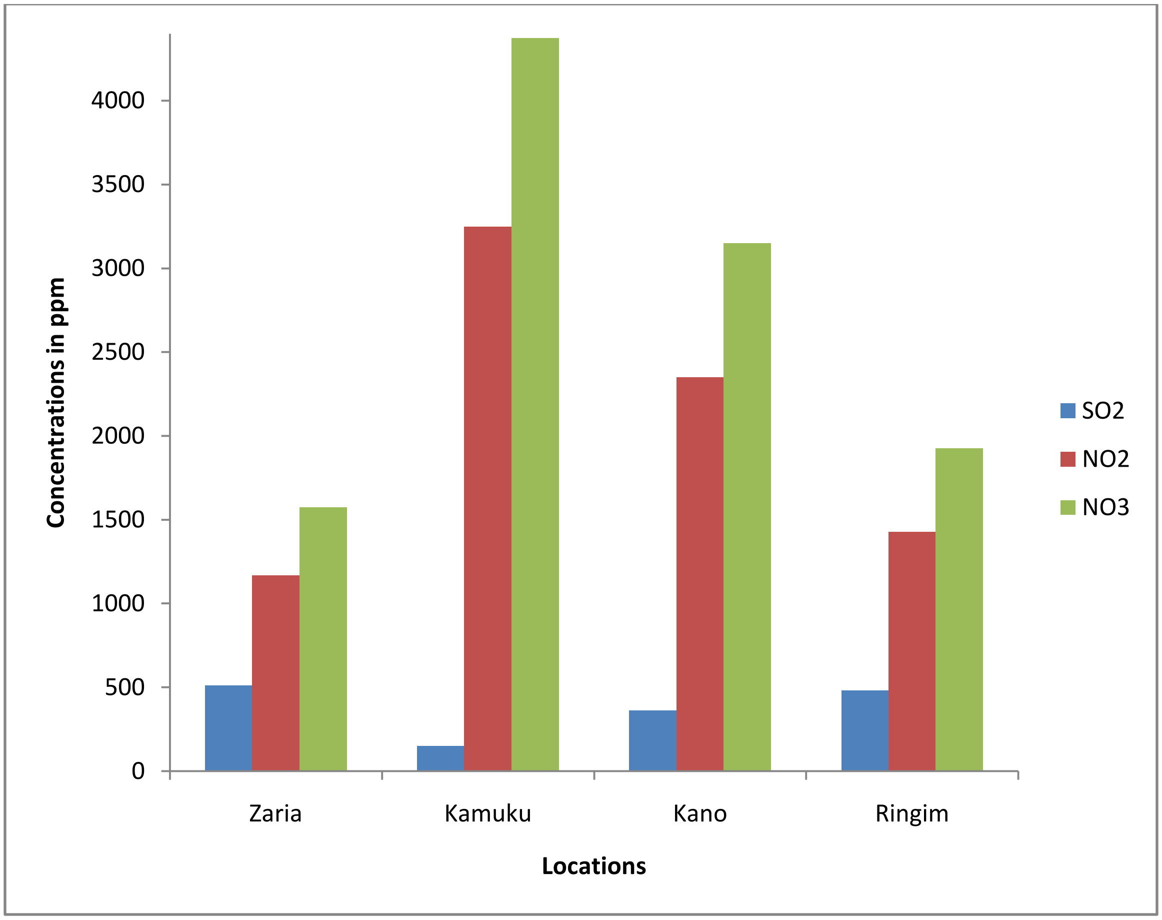

Fig. 4.10 Comparison of SO_2, NO_2 and NO_3 concentrations in different locations

The seasonal variation in the mean levels of SO_2, NO_2 and NO_3 concentrations was given in Table 4.9. There was higher accumulation of NO_2 and NO_3 in the wet season than the dry season, and the reverse in the case of SO_2 but statistically not significant in both cases with $p > 0.05$ as indicated in the table.

Table 4.9 Seasonal variation in the mean levels of SO_2, NO_2 and NO_3 concentrations (ppm)

	Dry	Wet	P value	Remark
SO_2	439.74±20.29	371.54±30.50	0.64	NS
NO_2	1342.86±155.24	855.39±55.02	0.22	NS
NO_3	1797.14±231.12	888.46±85.43	0.22	NS

NS=Not significant ($p \geq 0.05$)

4.6 Mean Levels of SO_2, NO_2 and NO_3 Concentrations in Different Moss Species

The mean levels of SO_2, NO_2 and NO_3 concentrations in different moss species were summarized in Table 4.10. The result statistically showed a significant difference in the uptake and accumulation of SO_2 by different moss species ($p < 0.05$). The accumulation of SO_2 was highest in *Barbula lambarenensis* and *Erpodium pobeguinii*, while the least recorded in *Hyophila crenulata*. Highest concentrations of NO_2 and NO_3 were recorded in *Fissidens grandifolius* followed by *Erpodium pobeguinii*, the least concentration of NO_2 recorded in *Fabronia pilifera* and least of NO_3 in *Brachymenium leptophyllum*.

Table 4.10 Mean levels of SO_2, NO_2 and NO_3 concentrations (ppm) in different Moss species

	SO_2	NO_2	NO_3
H. crenulata	150.94 ± 30.18^{e}	3250 ± 650^{c}	4375 ± 875^{c}
B. lambarenensis	513.2 ± 30.20^{a}	1170 ± 130^{d}	1575 ± 175^{e}
B. coronatum	362.25 ± 60.37^{c}	1815 ± 255^{d}	2450 ± 350^{d}
S. subjulaceum	241.51 ± 120.75^{d}	1690 ± 130^{d}	2275 ± 175^{d}
B. leptophyllum	275.35 ± 33.85^{d}	1820 ± 780^{d}	1225 ± 175^{e}
E. pobeguinii	513.2 ± 30.20^{a}	5845 ± 3545^{b}	7865 ± 5135^{b}
F. pilifera	422.63 ± 60.37^{b}	920 ± 120^{d}	1750 ± 700^{de}
F. grandifolius	356.67 ± 126.34^{c}	23630 ± 11810^{a}	31675 ± 19575^{a}

Values with same superscripts along the columns are not significantly different ($P \geq 0.05$)

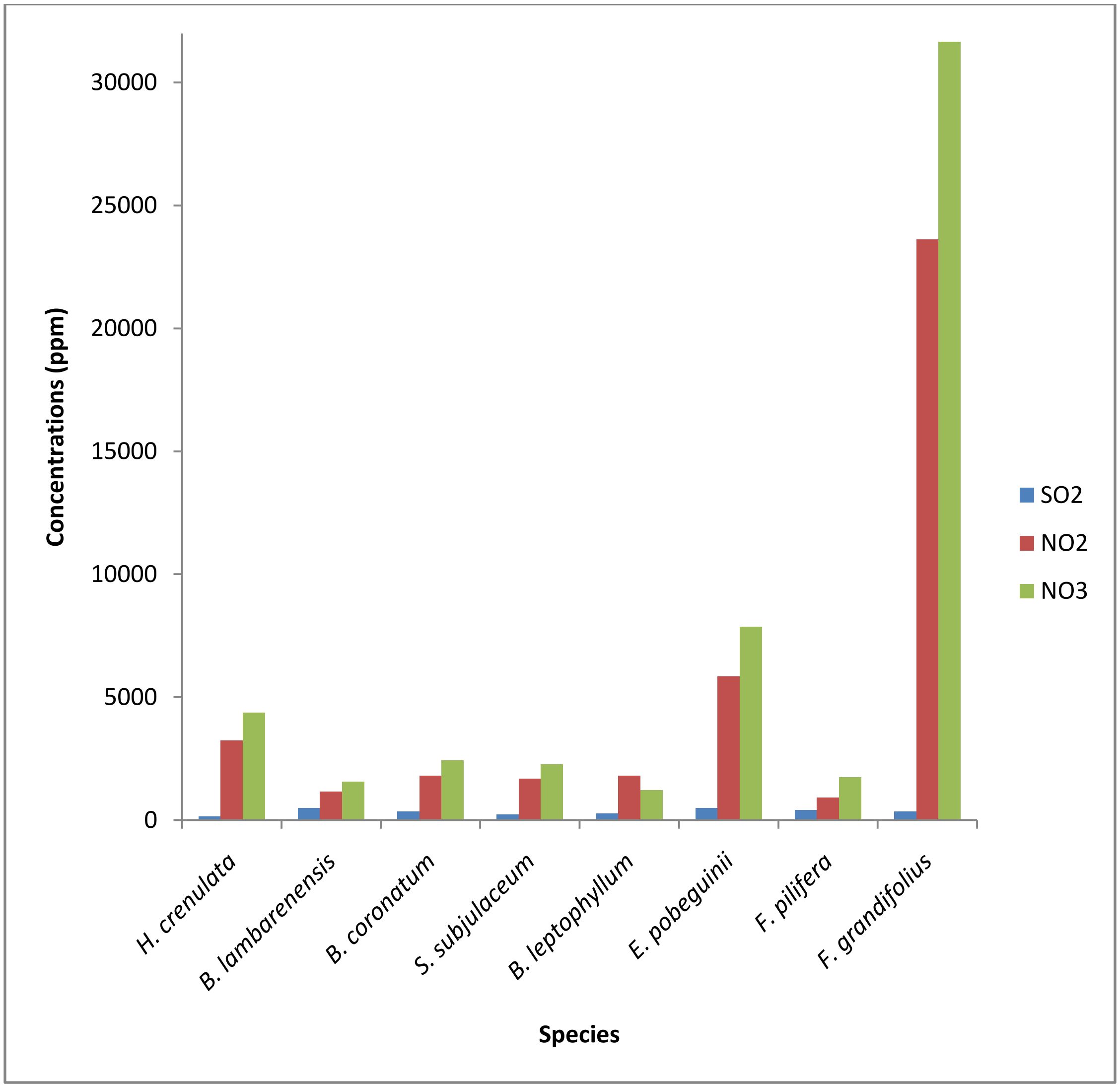

Fig. 4.11 Comparison of SO_2, NO_2 and NO_3 concentrations in different Moss species

CHAPTER FIVE

5.0 DISCUSSION

5.1 Distribution/Occurence of Bryoflora

Different species of moss plants including *Hyophila crenulata* C. Mull. ex Dus; *Barbula lambarenensis*; *Bryum coronatum* Schwaegr; *Splachnobryum subjulaceum* Card.; *Brachymenium leptophyllum*; *Erpodium pobeguinii* Par & Broth; *Fabronia pilifera* Hornsch and *Fissidens grandifolius* Broth & P. Varde. were found growing on different substrates including bark of trees, sandcrete materials (plastered and un-plastered walls, blocks, rock surface) and sand/soil/forest floor. Out of the eight (8) species recorded, *Hyophila crenulata* had the highest percentage occurrence, followed by *Erpodium pobeguinii, Barbula lambarenensis* and *Bryum coronatum* while the least percentage of occurrences was recorded in *Fissidens grandifolius*. This may be attributed to the fact that they were able to survive in almost all the substrates especially *H. crenulata* which was observed in all the substrates studied. It could also be due to differences in pH values and nature of the substrates, and many more.

The results tend to agree with the work of Adebiyi and Oyeyemi (2013) that revealed the occurrence of 8 different moss species in Ekiti State, Nigeria, which were distributed among 4 different substrates with *Bryum coronatum, Barbula lambaranensis, Thudium gratum* and *Hyophila excurrentinervis* having higher occurrences than others. It is also in agreement with Fatoba (2000) who collected moss samples from sandcrete materials and was able to reveal the presence of *Hyophila excurrentinervis,* *Bryum coronatum* and *Barbula lambaranensis* only.

Though, bryophytes occupy a wide range of habitats/substrates with respect to availability of moisture, their occurrence on these habitats/substrates could be attributed to suitable pH (Fatoba,1983) and availability of propagules at the time of exposure to bare soil or substrate (Banfield, 1976). Abubakar and Abdullahi (2012) carried out a study on Host preference of bryophytes composition from Northern Nigeria, where host specificity was shown by the recorded species in which pH value accounted for the marked variation in composition. According to them, *Erpodium coronatum* (Hook f. Wilson) Mitt. was the most abundant epiphyllous moss while *Fissidens glauculus* C.Mfill. was noted to be growing on a particular tank wall substrate. This indicates that mosses require specific substrates for occurrence or growth.

All the eight species were seen during the wet season, but only *Hyophila crenulata*, *Barbula lambarenensis* and *Erpodium pobeguinii* were observed during the dry season. This may probably be due to higher availability of moisture in the wet season. Bryophytes are sensitive to natural fluctuations in humidity. They dry out very quickly, but they can also absorb minute quantities of available moisture from fog, mist, and dew – sources of water that other plants cannot utilize (Hallingback and Hodgetts, 2000). However, during dry days there may be little physiological activity, and during droughts all physiological processes are quickly reduced to a minimum. Reproduction is highly dependable on water availability as the spermatozoids (male gametes) must swim from the antheridia to the archegonia in order to fuse with egg cells, initiating the spore-producing capsule generation, drought hampers this process.

The percentage occurrences of all the moss species in all locations indicate more species in Zaria and Kamuku than Kano and Ringim. *Hyophila crenulata* and *Bryum coronatum* were the species found across all the four locations, while others were distributed within Zaria and Kamuku. Higher number of species in these two locations could probably be as a result of high levels of atmospheric humidity and low rates of evaporation, which favors the growth and reproduction in the bryobiotina subkingdom. Also, the regions have relatively greater number of trees compared to Kano and Ringim which tend to have very few scattered trees and drier environments, thus favoring the growth of many bryophytes that grow only on sandcrete substrates. According to Abubakar and Abdullahi (2012), the present-day changing environment due to human activities of logging for forest resources and in our traditional African practices of firewood harvesting is a major limitation to the conservation of our natural bryoflora diversity. Absence of some species in Kano may also be attributed to higher concentrations of most of the pollutants in the area. Bako *et al*. (2008) carried out a survey on the spatial distribution and heavy metal content of some bryophytes and lichens in relation to air pollution where they indicated the number of species (diversity) for both the bryophytes and lichens to be increasing as one moved away from the source point. Similarly, abundance as estimated by percentage of tree surfaces covered by epiphytes, increased from rare to abundant as one moved away from the source point.

5.2 Mean Levels of Heavy Metal Concentrations in Different Locations

Kano was having the highest mean concentrations of 4 of the 7 heavy metals analyzed which are Cu, Cd, Cr and Pb; Zaria having the highest concentration of Zn while Kamuku the highest of Co. The comparison and test of significance of the elemental concentrations indicated that Kano is relatively more contaminated with almost all the heavy metals investigated, followed by Zaria and then less contamination in Kamuku and

Ringim. Higher concentrations of the various pollutants in Kano may be as a result of substantial number of industries compared to other locations, heavy traffic and more human activities as it is one of the most populous cities in Nigeria. It is the center of commercial activities in the country. High mean values of the metals in the area may be due to the high emission of these metals in the air. Shakya *et al*. (2001) carried out a study on the appraisal of some mosses for biomonitoring airborne heavy metals in Kathmandu Valley situated in the middle part of the Himalayan range. They indicated the most severe contaminated sites with Cd, Cr and Pb to be the areas with high vehicular movement and high population density. This is also in agreement with the work of Chen *et al*. (2010) who compared heavy metal accumulation capacity of some indigenous mosses in Southwest China (a case study of Chengdu city). Their conclusion was that atmospheric pollution of heavy metals in Wangjiang Park was relatively more serious than that of Ta Zishan Park and Cultural Park, which may be result of steel industry, heavy traffic and more human activities near Wangjiang Park.

Some researchers also indicate that environmental characteristics such as climatic conditions, mineral composition of soil dust, soil water, natural element cycling process and vegetation zone may have a significant influence on uptake efficiencies of heavy metal elements in moss (Ross, 1990; Zechmeister, 1998; Reimann *et al*., 2001, Chakrabortty *et al*., 2006). Kano, compared to Zaria and Kamuku has low level of atmospheric humidity and higher rate of evaporation which affect the growth and reproduction in the bryophytes and may lead to higher accumulation of the pollutants.

Heavy metals originate from both natural and anthropogenic sources in the environment. In the atmosphere, natural sources of these elements are volcanic eruptions, cosmic and terrestrial dusts, vegetation fires, and salt spray from the oceans. Anthropogenic sources include emissions from different industrial plants (steel and non-ferrous metallurgy, smelters, alloying plants, petrochemical industry, fertilizer plants, coal power plants, industrial and home furnaces) and motor traffic (Zechmeister *et al*., 2003). The amount of heavy metals originating from natural sources in the atmosphere is small compared with the anthropogenic flux of these elements (Zechmeister *et al*., 2003)

In general, the mean concentration of Zn was highest and that of Cd was the lowest in all locations. The total average abundance of each metal and in each location was in the following order: Zn > Cr, Cu, Pb > Ni > Co > Cd. This finding is similar to that of Chen *et al*. (2010) who showed the total average abundance of each element in the following order: Fe > Zn > Mn > Cu > Pb > Cr > Ni > Cd in a study carried out in Chengdu city. There are no significant differences in the accumulation of a wide range of trace metals (e.g. Pb, Cd, Cu, V) between the mosses *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) B.S.G., according to Halleraker *et al*. (1998). Some researchers also indicate that environmental characteristics such as climatic conditions, mineral composition of soil dust, soil water, natural element cycling process and vegetation zone may have a significant influence on uptake efficiencies of heavy metal elements in moss (Ross, 1990; Zechmeister, 1998; Reimann *et al*., 2001, Chakrabortty *et al*., 2006).

The unusually elevated concentration of Zn in Zaria may be attributed to the presence of large crop fields and agricultural activities where spraying is usually undertaken especially around PZ area almost throughout some times by irrigation, as no any other possible source was observed around the sampling area. This agree with the work of

Shakya *et al.* (2001) that observed high mean value of Zn outside the ring road in southeastern part of Kathmandu Valley and related that to the agricultural activities around the Valley. Even though Kamuku is relatively with less anthropogenic activities, Co concentration was somewhat high which is probably due to its proximity to the only highway that links Northern and Southern part of the country always with large vehicles conveying goods and passengers.

5.3 Seasonal Variation in the Levels of Heavy Metal Concentrations in Different Locations

Ni, Cu, Cd, Cr and Co showed relatively higher accumulation values in the wet season than the dry season, while Pb and Zn had higher values in the dry season but statistically not significant in all cases. This could probably be as a result of the moss plants accumulating the pollutants continuously throughout for somewhat long period in them, in which rain is not likely to be washing what is already inside them. In a study carried out in Galicia (Northwest Spain) using moss for biomonitoring metal deposition by Fernandez and Carballeira (2002), no significant differences were found in the concentrations of all the elements studied among the seasons, just as the findings in this work. Bryophytes possess a number of characteristics that make them suitable for use as biomonitors of air pollutants. They are usually very long lived and their morphology does not vary with season (Chakrabortty *et al.*, 2006). The result is also in agreement with the work of Onianwa *et al*. (1986) on accumulation pattern of heavy metals in forest mosses from south west region of Nigeria. They concluded that comparison of data on wind and wet precipitation for parts of the region with the metal accumulation gradients did not show any correlation. However, it contradicts the findings of Ekpo *et al*. (1012) whose results showed the mean levels of trace metals to be higher in the wet season than in the dry season.

5.4 Mean Levels of Heavy Metal Concentrations in Different Moss Species

A comparison of the elemental concentrations in the moss species was carried out to see their differences in accumulation and uptake efficiency of the heavy metals, and a significant difference was observed among the different moss species studied. *Bryum coronatum* was found to have the highest concentrations of four (4) of the seven (7) analyzed metals which are Cu, Pb, Cr, and Zn; *Fissidens grandifolius* having the highest concentrations of Ni and Co while *Fabronia pilifera* with the highest concentration of Cd. *Hyophila crenulata* had the second highest concentrations of Cu, Pb, Cr and Co. There was no accumulation of Cr and Co recorded in *Fabronia pilifera*.

Variations in the level of accumulation of individual metals in moss species could be as a result of differences in their morphological and physiological characteristics and growth habit. Interspecies comparison of specific heavy metal contents by Chen *et al*. (2010) indicated that the biological characters such as living form and morpha had a great influence on accumulative capacity even if collected from the same biotope. Most moss species had different accumulation capacity even for the same heavy metal element. It could also be a function of the differential ion exchange potentials of the species, with particular reference to the cation exchange capacity and formation of chelates by the metals (Crist *et al*., 1996; Zechmeister *et al.*, 2003; Chakrabortty *et al.*, 2006). The attachment of the particle is affected by the size of the particle and the surface structure of the mosses. Ion exchange is a fast physiological-chemical process that is affected by the number and type of free cation exchange sites, the age of the cells, their reaction to desiccation, growing condition, temperature, precipitation, pH, composition of the pollutants, and leaching (Tyler, 1990). In the ion exchange process, cations and anions become attached to the functional organic groups in the cell wall primarily through chelation (Rao, 1984).

The high bioaccumulation of heavy metals by *Bryum coronatum, Hyophila cenulata,* and *Fissidens gandifolius* may be due to their morphology and growth form, in that their leaves are compactly arranged, growing in turfs, which must have facilitated them to trap different suspended particulate matters. The dense canopy of mosses acts as an efficient filter in trapping airborne particles and ultimately higher element concentrations (Herpin *et al*., 2001). Markert *et al*. (1996) listed some moss species such as *Bryum radiculosum, Aloina aloides, Tortella flavovirens, Scleropodium purum* and *Polytrichum formosum* selected for biomonitoring of atmospheric pollution in the Czech and Slovak Republics. Studies conducted in and around Mumbai, a major metropolis in western India, have identified *Pinnatella alopccuroides, Pterobryopsis flexiceps* and *Bryum* spp. as effective biomonitors for the region (Chakrabortty *et al*., 2006).

There are natural differences in chemical composition between individual species with different growths and conditions, and between separate parts of the individual moss. There are natural differences in chemical composition between individual species and even among populations of the same species, between individuals with different growth and conditions, and between the separate parts of the individual moss (Thöni *et al*., 1996).Theoretically, biomonitoring species for trace-element air pollution are selected on the basis of specificity (accumulation is considered to occur from the atmosphere only)

(Rühling, 1994). In practice, to be a suitable biomonitor some specific requirements have to be met. Suitable biomonitors, which meet the requirements, make continuous monitoring and even retrospective monitoring of air pollution possible at relatively low cost. When information on time-averaged trace-element concentrations at specific sites in the environment is the aim, the use of such non-mobile monitors is preferred (Chakrabortty *et al*., 2006).

There was no accumulation of Cr and Co recorded in *Fabronia pilifera*. It may be that the species is having low affinity to such metals. The variation in the accumulation pattern of the heavy metals among the different species might be due to the differences in binding affinity of the metals to the moss species (Rasmussen, 1978). In different moss species, cations are absorbed by different molecules in cell walls, for example in *Sphagnum* mosses by polygalacturonic acids and in liverworts by mannuronic acids (Brown, 1982). It might thus result different affinities of mosses for different heavy metals even within the same genus due to the varying cell wall contents. One can also predict the suitable moss species that may be used as a biomonitor for a single trace element, or a group of trace elements. It is very difficult for any other approach to obtain such a detailed picture of variations in time and space at a reasonable cost (Chakrabortty and Paratkar, 2006).

Zn concentration was highest than other heavy metals in all the species while Cd was having the least concentration in all. The reason for lowest concentration of Cd in all cases might be that it is a trace element (Chen *et al*., 2010). In general, the trend of accumulation in the moss species was in the following order: Zn > Pb, Cr, Cu > Ni > Co > Cd which is in agreement with the findings of Chen *et al*. (2010).

5.5 Mean Levels of SO_2, NO_2 and NO_3 Concentrations in Different Locations

The concentrations of SO_2, NO_2 and NO_3 all vary significantly both within and among the study locations with P-Values < 0.05. The highest concentration of the SO_2 was observed in Zaria and the least in Kamuku, while highest concentrations of NO_2 and NO_3 were found in Kamuku and least in Zaria. High concentration of SO_2 in Zaria may be attributed to relatively high traffic density coupled with agricultural activities taking place where fertilizers and various chemicals are sprayed almost throughout the year in some areas adopting irrigation system of farming such as the PZ site. The case may be the same with NO_2 and NO_3 in Kamuku which is located near the busy road connecting Northern and Southern part of the country. Abam and Unachukwu (2009) report the results of the investigation of vehicular emissions in selected areas in Calabar Nigeria, monitoring various parameters such as CO, NO_2, SO_2 and noise level. The highest concentrations of NO_2, SO_2 and noise level were at sampling point 2 (SP2) where according to them traffic intersections and traffic count is high. Environmental factors associated with nitrate accumulation drought, high temperature, cloudy weather, low availability of nutrients, damage by insects and herbicides, and excessive soil nitrogen (Barbara *et al*., 1975).

Sulphur dioxide originates from both natural and anthropogenic sources in the environment. Natural sources of the sulphur in the atmosphere are volcanic activity, fires and bacterial activity, the sea and ocean. The main sources of anthropogenic emissions of sulphur are the energy sector and various factories where fossil fuels are used, especially coal and oil (Zechmeister *et al*., 2003). The main anthropogenic sources for oxidised forms of nitrogen are transport, industry and energy production, estimated to contribute up to 70% of oxidized nitrogen emissions (Bragazza *et al*., 2005). Additional sources include soil emission, particularly under high nitrogen inputs. Emission sources of reduced forms of nitrogen are primarily related to agricultural activities such as animal husbandry and the application and production of fertilizers. Another important source of nitrogen emission is forest fires (Jovan and Carlberg, 2007).

Sulphur Dioxide is emitted directly into the atmosphere and can remain suspended for days allowing for wide distribution of the pollutant. One effect of sulphur dioxide is the formation of acid rain (Adoki, 2012). According to him, Sulphur dioxide in the air is hazardous to vegetation, and that high SO_2 emission level is due to some extent from automobile emissions. At ambient concentration levels, NO_2 is an irritating gas that may constrict the airways of asthmatics. The total nitrogen concentration in mosses can potentially be used as a surrogate to estimate total nitrogen deposition and identify areas with high nitrogen deposition at a high resolution (Harmens *et al*., 2008).

5.6 Seasonal Variation in the Levels of SO_2, NO_2 and NO_3 Concentrations in Different Locations

There was higher accumulation of NO_2 and NO_3 in the wet season than the dry season, and the reverse in the case of SO_2 but all are not statistically significant. The reason may be similar to that in case of heavy metals above, that moss plants accumulate the pollutants in them continuously for somewhat long period and the rain is not likely to be washing what is already inside the plants. Bryophytes are usually very long lived and their morphology does not vary with season (Chakrabortty *et al.*, 2006).

5.7 Mean Levels of SO_2, NO_2 and NO_3 Concentrations in Different Moss Species

The results showed a significant difference in the uptake and accumulation of SO_2, NO_2 and NO_3 by different moss species. The accumulation of SO_2 was higher in *Barbula lambarenensis* and *Erpodium pobeguinii*, while the least recorded in *Hyophila crenulata*. Highest concentrations of NO_2 and NO_3 were both recorded in *Fissidens grandifolius* followed by *Erpodium pobeguinii*, the least concentration of NO_2 was recorded in *Fabronia pilifera* and that of NO_3 by *Brachymenium leptophyllum*. These may be as a result of differences in their morphological and physiological characteristics and growth habit. Different researchers concluded that morphological structure and nature of growth form affect the pattern and rate of accumulation of various atmospheric pollutants by moss plants such as Zechmeister *et al*. (2003), Harmes *et al*. (2008) and Chen *et al*. (2010). Plant associated factors that influence nitrate accumulations include plant species variability, selective localization within the plant, stage of maturity and nitrate reductase activity (Barbara *et al*., 1975).

Another reason for difference in uptake ability may be differential ion exchange potentials of the species, with particular reference to the cation exchange capacity. Ion exchange is a fast physiological-chemical process that is affected by the number and type of free cation exchange sites, the age of the cells, their reaction to desiccation, growing condition, temperature, precipitation, pH, composition of the pollutants, and leaching (Tyler, 1990). In the ion exchange process, cations and anions become attached to the functional organic groups in the cell wall primarily through chelation (Rao, 1984).

The total nitrogen concentration in mosses can potentially be used as a surrogate to estimate total nitrogen deposition and identify areas with high nitrogen deposition at a high resolution. A pilot-study in Scandinavian countries showed a strong linear relationship between the total N concentration in mosses and the European Monitoring and Evaluation Programme (EMEP)-modelled atmospheric N deposition rates (Harmens *et al*., 2005). Therefore, for the first time in Europe heavy metals in mosses survey the total N concentration was also determined in naturally-growing mosses in about 60% of the participating countries in 2005/6 (Harmens *et al*., 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS.

6.1 Summary

Eight (8) different species of moss plants were seen/observed in this present study, which are *Hyophila crenulata* C. Mull. ex Dus; *Barbula lambarenensis*; *Bryum coronatum* Schwaegr; *Splachnobryum subjulaceum* Card.; *Brachymenium leptophyllum*; *Erpodium pobeguinii* Par & Broth; *Fabronia pilifera* Hornsch and *Fissidens gandifolius* Broth & P. Varde. Out of these, *Hyophila crenulata* and *Bryum coronatum* were found across all the four locations.

The results of the present investigation confirmed the presence of heavy metals (Co, Ni, Cu, Cd, Pb, Cr, and Zn) as well as SO_2, NO_2 and NO_3 in the environments of all the study locations, at the time of sampling. There was significant variation in the concentration of all the pollutants studied both within and among the locations and moss species. This was shown to be as a result of differences in anthropogenic activities. Kano was having the highest concentrations of almost all the major pollutants, followed by Zaria, due to availability of more sources of the pollutants such as automobiles. In general, the mean concentration of Zn was highest and that of Cd was the lowest in all locations. The total average abundance of the metals in each location was in the following order: Zn > Cr, Cu, Pb > Ni > Co > Cd.

From the study, it can be said that out of the different mosses studied, three species *Bryum coronatum, Fissidens grandifolius* and *Hyophila crenulata* were precise and useful bioindicators/biomonitors of heavy metals in the studied locations. Among them *B.*

coronatum is the good accumulator of majority of the metals. SO_2 accumulation was better in *Barbula lambarenensis* and *Erpodium pobeguini*, while *Fissidens grandifolius* was better in accumulating NO_2 and NO_3. There is no significant variation in accumulation of all the studied pollutants between wet and dry seasons. The results provide baseline information for regional atmospheric pollution monitoring using moss species as effective biomonitors in the studied locations.

6.2 Conclusions

Heavy metals (Co, Ni, Cu, Cd, Pb, Cr, and Zn) as well as SO_2, NO_2 and NO_3 were present in the environments of all the study locations, at the time of sampling.

There was significant variation in the concentrations of all the pollutants both within and among locations and moss species. Kano had the highest concentrations of almost all the major pollutants, while Ringim had the least.

Bryum coronatum, *Fissidens grandifolius* and *Hyophila crenulata* were precise and useful bioindicators/biomonitors of heavy metals in the studied locations. Among them *B. coronatum* was a good accumulator of majority of the metals.

SO_2 accumulation was better in *Barbula lambarenensis* and *Erpodium pobeguini*, while *Fissidens grandifolius* better in accumulating NO_2 and NO_3.

There is no significant variation in accumulation of all the studied pollutants between wet and dry seasons.

The results provide baseline information for regional atmospheric pollution monitoring using moss species as effective biomonitors in the studied locations.

6.3 Recommendations

The study recommends the use of *Bryum coronatum, Fissidens grandifolius* and *Hyophila crenulata* or *Bryum coronatum* alone for biomonitoring program in the Northwest region of Nigeria.

It is also recommended that pollutants in the environment should be investigated and monitored from time to time, as it is done in developed countries, considering the toxicological risks posed by them to human health in particular and the environment at large.

The use of bio-fuel, closure of heavy industries located near residential areas, emission control legislation, reduction in fossil fuel combustion and significant decrease in leaded petrol combustion should be encouraged for the safety of our dear environment.

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APPENDICES

APPENDIX I

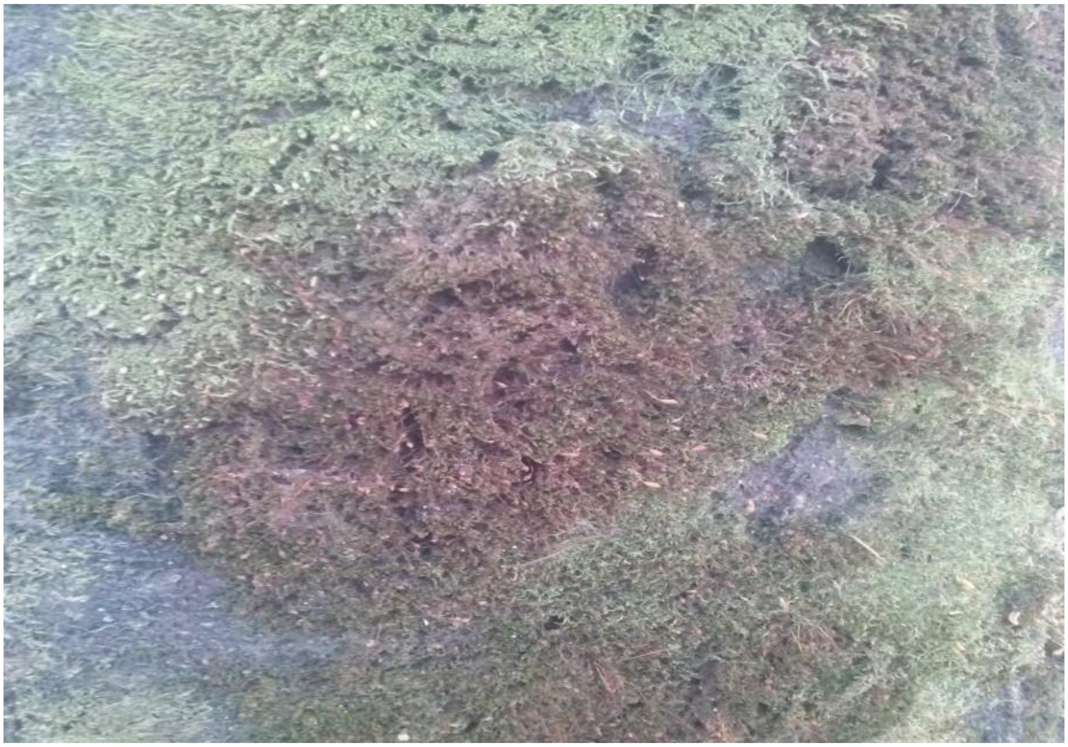

Plate 1: *Erpodium pobeguiini*

APPENDIX II

Plate 2: *Barbula lambarenensis*

APPENDIX III

Plate 3: *Bryum coronatum*

APPENDIX IV

Plate 4: *Hyophila crenulata*

APPENDIX V

Plate 5: *Fissidens grandifolius*