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Determination Of Some Concentrated Atmospheric Pollutants Such As Heavy Metal (So_2 And No_3) In Lichens Found In Urban And Peri-Urban Areas Of Kaduna State.

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ABSTRACT

Atmospheric deposition of heavy metals like SO_2 and NO_3 was investigated using lichens as suction points. The lichens samples were collected from four study areas namely; ABU Botanical garden, PZ area, (Sabon Gari Local Government) Buruku and Rido village (Chikun Local Government). Samples were collected in two seasons, that is three months in Dry season and three months in Wet season, the sample were collected 300m away from the main road. The representative samples were wet digested in Hydrogen trioxonitrate (HNO_3) and Hydrochloric acid (HCL). Heavy metals content were measured in the extract using Atomic Absorption Spectrometry (AAS); Quantitative analysis of the samples was carried out using Sequential Atomic Absorption Spectrometry (Varian AAS 240FS). Determination of Nitrogen Oxides (NO_3) was carried out using Phenoldisulphate acid method. Sulphur dioxide (SO_2) was determined at 420nm using Bosch and Lomb Spectronic-70. Data obtained was analyzed statistically using Analyses of variance (ANOVA), Duncan's multiple range test (DMRT) was used to separate the means where there is significant differences. The species of lichens collected are*; Phaeophyscia* species, *Xanthoparmelia* spp, *Flavopermelia capirata, Dictyonema glabratum,* and *Physcia* species. There is significant difference among the lichens in the accumulation of all the pollutants and *Flavopermelia capirata* has higher accumulation of the most pollutants. The results from all the locations shows that, PZ area is significantly higher in SO_2, Rido is significantly lower in NO_3 concentration. Also, for the seasonal variation dry season (Jan., Feb., Mar.) shows high content of NO_3 (0.099mg/L) and SO_2 (355.343mg/L). The concentration of Nitrogen oxide NO_3 in lichens ranged from 0.14 – 0.05mg/L while that of Sulphur dioxide SO_2 ranged from 238.99 – 62.89mg/L. Data Obtained reveal the important contributions towards understanding of heavy metal deposition patterns and provide baseline data, that can be used for identification of areas potential at risk from atmospheric pollutants contamination in the areas and seasons. The use of epiphytic lichens can provide a cost-effective approach for monitoring atmospheric pollutants contamination and may be effectively used in large scale air pollution monitoring programmes.

Keywords:Atmospheric, pollutant, Heavy Metals, lichen, Urban, Peri-Urban, Kadunna

INTRODUCTION

Lichens

The word 'Lichen' (lie ken) was introduced into the Greek literature in about 300 BC by Theophrastus, to describe outgrowths from the bark of olive trees [1]. Lichens are composite organism which are form as a result of symbiotic association between fungus (the mycobiont) with a photosynthetic partner (the photobiont), usually either a green algae or cyanobacterium [2]. The intimate association of these two microorganisms results in the formation of a macro-organism, i.e. the lichen thallus whose morphology is quite different from that of the original organisms. The association between the algae and fungus is so intimate that, the term symbiosis is often applied to it for its description. Lichens form easily distinguishable coloured patches on tree barks, rocks and soil and are universally distributed organisms occurring in varied climatic conditions ranging from the poles to the tropics [3].

Despite the understanding of its important roles in the ecosystem, the study of lichens remains quite neglected throughout the world, though they together with mosses form the dominant organisms in ecosystems covering over 10% of the earth terrestrial habitats, particularly at higher elevations [4]. Lichens were the first examples of symbiosis in which the translocation process was demonstrated especially the movement of carbohydrates from algae to fungi. They have evolved with ability to absorb minute concentrations of water from air or dew and become metabolically active within a few minutes, whereas inversely in scorching sunny conditions, they lose water and become dry and crisp within an hour [5]. The Mycobiont part of the Lichen form attachment with substratum and also aids in the absorption of moisture, micro and macronutrients for the photosynthetic partner to grow and in turn synthesize the carbohydrates for their metabolic activity. This constant supply of carbohydrates enables the fungal partner to continuously grow and reproduce, unlike the free living fungi that appear only upon the availability of moisture and nutrients. Lichens traditionally have been considered a type of fungus due to its dominance of the association. Lichens have diversified extensively in the past 600 years, and occur over more than 10% of the terrestrial surface [6]. About 13,500 species are currently accepted and it is estimated that the actual world total will be in the range of 17,000 - 20,000 [7]. They form an integral and important part of an ecosystem and serve as ecological indicators too. They are included in the lower groups of plants known as the Cryptogams which reproduce by the means of spores and do not produce seeds. It is believed that the algae, which belong to those families of Chlorophyceae and Myxophyceae that lived on dry land and had become aerial before their association with fungi to form lichens [8].

The fungal hyphae can combine with a considerable number of different algae, so that, even as regards the algal symbiont, lichens are truly polyphyletic in origin. Two groups of fungi associated with algae forming lichens are; Basidiomycetes found in only a few genera and Ascomycetes which associate with the various algae and form a bulk of lichen families. Though, lichens have no common origin, they are fitted for much longer existence than that of fungi and can persist through extreme climatic changes [9]. The main plant body of the lichen is a vegetative portion known as thallus which is comparable to the vegetative portions of other cryptogams such as mosses and liverworts. The fungal component (mycobiont) is an Ascomycete or Basidiomycete which forms a symbiotic relation with green algae or blue green algae (phycobiont). After this association, both the phycobiont and mycobiont components lose their individuality and the lichen behaves as a single organism, both morphologically and physiologically [10]. In lichen thallus (body) the mycobiont predominates with 90% of the thallus volume and provides shape, structure and colour to the lichen with partial contribution from algae and hence, the lichens are placed in the kingdom – Mycota [11]. Lichens have highly organized thallus than corresponding non-lichenized Ascomycetes and also produce vegetative structures not known in other fungi [12].

Lichens are characterized by a variety of vegetative structures on the upper and lower surfaces of the thallus. The colour of the thallus, texture (smooth, rough, warty), presence of finger like projections (isidia), granular powder in groups (soredia), fine powder (pruina), black dots (pycnidia) and whitish decorticated areas (pseudocyphellae) are to be noted on the upper surface of crustose and foliose lichens. The colour of lower surface, presence of any pores, presence or absence of rhizomes (root like structures), their colour, distribution, branching, abundance is to be noted on the lower surfaces of foliose lichens [13.

Vegetative Classification of Lichens

Classification Based On Habitat

Based on the substratum of growth, Lichens can be broadly classified as – Corticolous (growing on the bark surface of trees), Follicolous (growing on the surface of leaves), Saxicolous (growing on rock surfaces), Terricolous (growing

on soil) and Musicolous (growing on mosses) [14]

i Corticolous Lichens

These develop on bark and contain fruticose and foliose species These include the species of *Evernia, Parmelia* and *Usnea*. Growth of lichens on tree bark depends on its stability, texture, pH and water retention ability. The rough barked trees encourage Parmelioid and Physioid lichens along with members of *Buellia, Lecanoraceae, Lecideaceae* and *Pertusariaceae*.

The rough bark help lichens in trapping their spores or vegetative diaspores and retains moisture for longer duration [15].

ii Follicolous Lichen

Species like *Calicium, Cyphelium* and *Strigula* occurring on leaves are called as follicolous lichens.The shiny, smooth evergreen leaves in outer canopy, shady understory, in light gaps and near water bodies provide suitable substratum for follicolous lichens [16].

iii Saxicolous Lichen

Lichen communities developed on rocky substratum are called Saxicolous and these vary according to rock type [17]. The type of rock and pH are important factor responsible for colonization of the rock by lichen communities. The species like *Caloplecta*, *Aspicilia* grow on hard lime stones. *Verrucaria* species can be seen on well lit areas. *Lepraria*, *Cystocoleus* community grows on siliceous rocks [18].

iv Terricolous Lichen

The lichens of this community are growing on the ground or soil and often form a dominant component of the ground vegetation in the extreme environments [19].

V Muscicolous Lichen

These lichens grow on mosses, Some species like *Cladonia*, *Peltigera* grow along with mosses. They prefer the rough and bushy nature of the mosses which are efficient in trapping the lichen propagules. The hygroscopic nature of the mosses provides better water relation and micro-climatic niche to the lichens growing on them [20].

Classification Based On Growth Forms

The growth forms are usually conspicuous on the substrates, forming grey, green or even orange patches and are categorized primarily based on their morphology and size into three major types viz. Crustose (crust like), foliose (leaf like) and fruticose (shrubby) [21]. The lichens belonging to the first category are usually called microlichens and the latter two are referred to as macrolichens [22].

i Crustose Lichens

These types of lichens lack an organized thallus and are closely attached to the substratum. They consist of an indeterminate hyphal mat which entraps and encloses algal colonies. Such rudimentary thalli occur in the lower species of *Calicium, Pyrenula*, *Trypethelium*, *Xylographa* and *Arthonia* [23]. Majority of crustose lichens like species of *Lecanora* and *Lecidea* grow on the surface of rocks and trees with distinct thallus [24]. The surface is often warty or the entire thallus is marked off into many-sided areas or areoles and is therefore spoken of as areolate. The highest stage in development of crustose lichens is squamulose thallus. In this type the individual lobes still lacking a lower cortex become partially free of the substrate [25]. Soil lichens like *Cladonia*, *Catapyrenium*, *Psora* contains this type of thallus.

ii Foliose Lichens

They are also called as leafy lichens. The thallus in this case is loosely attached to the substratum by rhizines with distinct upper and lower surfaces. The thallus is typically divided into branching lobes as in *Heterodermia*, *Physcia*, *Xanthoria*, *Cetraria* and *Parmelias*. The foliose type of lichens merges into the fruticose type in the ascending series and into the crustose type in the descending series [26].

iii Fruticose Lichens (Shrubby)

These are hair like, shrubby, finger like or strap shaped. Here the lichen thallus is attached to the substratum at one point and the remaining major portion is either growing erect or hanging [27]. These vary in size from minute *cladonia* spp (only 1-2 mm) high to strands of *Usnea* spp (up to 5 m long). The internal structure is radial with a dense outer cortex, a thin algal layer, a medulla and more or less hollow centre or a dense central cord. The thallus is round or flattened and richly branched. It is attached at the central or basal point known as the umbiculus, which consists of a hyphal tissue holding the plant firmly attached to the substratum and

taking there from moisture and soluble food-substances [28].

Lichen Ecology and Distribution

The symbiotic relationship helps the lichens to live in variety of habitats and climatic conditions all over the world including extreme environments. Within a climatically uniform region each particular substrate tends to assume eventually a characteristic and often remarkably uniform lichen community [29]. They grow in diverse climatic conditions and on diverse substrates. The ability to quickly absorb and retain water from many sources makes it possible for lichens to live in harsh environments like deserts and polar regions, and on exposed surfaces like bare rocks, walls, roofs, tree branches and man-made substrata like glass, metals etc. They occur in virtually every pioneer terrestrial habitat from Arctic and Antarctic to tropical areas and in many desert areas where they are able to form long lived and stable communities [30]

Since preference for habitats and microhabitats is well-developed in lichens, small differences in chemical (pH and mineral contents) and physical factors (light, temperature, humidity, wind, substrate porosity, toughness and roughness) can explain species replacement. [31] also studied factors that influence species composition of epiphytic lichens, have concluded that, the most important factor was macroclimatic gradient followed by the spatial variation and substrate variation. Several researches revealed that, the microclimate has a greater influence on establishing epiphytic communities than the substrate, since the phorophyte is a non continuous variable, unlike the environmental ecological variables that usually establish gradients. [32] stated that, variations in the presence of corticolous lichens depends more on the physical nature of the bark than on tree species, thus, analyzed epiphytic lichens in oak forests and found a homogeneous lichen community on the sides in young trees, while on the older trees the community composition on the trunk sides are modified. According to other Scientists, these changes may be related to trunk roughness and micro-climate. The epiphytic community differs more strongly depending on trunk height, although a difference was also found in the trunk communities on trunks of different ages [33].

Thus, therefore the substrate structure and the physical environmental characteristics are among the principal factors affecting lichen distribution on tree trunks. The physical-chemical characteristics of tree bark, such as texture, hardness, water retention, pH, macro and micro nutrient composition are essential for the establishment of the lichen community [34]; [35]. Trees with smooth bark usually present only crustose forms, many of them with a very thin thallus. When the tree begins to age and the bark roughness, increases other forms of lichens appear, such as crustose species with thicker thallus or large foliose species, as well as fruticose ones. Factors such as tree age, exposure to sunlight and dust are of special importance for the kind of lichen community that will colonize tree trunks. Depending on the circumstances, this community may be poorer or richer than that on the twigs. Likewise, it may happen that, in more advanced stages, many bryophytes, especially mosses, form communities over wide areas, occupying the place of lichens [36].

Mechanism of association in lichens

Lichens are symbiotic organisms composed of a fungal partner (the mycobiont) and a green or blue-green algal partner (the photobiont) [37]; [38]. Symbiotic interactions are quite extensive and involve nitrogen metabolism, synthesis of secondary metabolites and the transfer of carbohydrates. Regarding lichen reproduction, many mycologists assume that, once a lichen fungal ascospore has contacted a suitable photobiont, or once a soredium, isidium or lichen fragment has landed on an appropriate surface, a lichen thallus will develop into mature lichen [39]. For germination, the fungal spores of most lichens do not need the photobiont: they are reported to grow and encircle any spherical structures of suitable size, including glass beads or rods [40]. In liquid cultures, however, the isolated fungus is reported to behave partly like yeast, producing large amounts of single cells, whereas the isolated algae show thickened cell walls [41].

The fungal component may comprise some 75% of the total lichen mass [42], but hardly anything is known about the precise participations of the symbiotic algae and fungi in metal accumulation [43]. Most commonly, photobionts are located in a layer within the fungal tissue. [40]. The layer is generally oriented in a manner that maximizes photosynthesis, and is protected from rapid changes in water availability. Each cell or group of cells of the photobiont is usually wrapped by hyphae, and in some cases penetrated by a haustorium. Moribund cells may be digested by the fungus, but for the most part, the photobiont remains healthy during the functional period of the symbiosis. The increased size of cells of the photobiont indicates that, reproduction is regulated by the symbiosis [38]. This photobiont is found within a layer below the surface of the lichen. Cyanobacteria may also be held in small eruptions of or under the surface called cephalopodia. Cyanobacteria can fix atmospheric Nitrogen, and thus, complement the primary activities of the photobiont, energy fixation. The thallus may be covered by or enmeshed in extracellular matrix expressed by the fungus. For instance, some crustose lichens have a polysaccharide layer on the surface, the photobiont is located at the base of the polysaccharide layer. Polysaccharide layers may also be found within the cortex of the thallus where their function may be different [41]. The thallus is commonly interleaved by hyphal layers. Some thalli have hydrophobic layers on the surface or within the thallus. The hydrophobicity appears to be related to the presence of hydrophobins expressed by the fungus. Indeed, different hydrophobins act in different parts of the thallus. Finally, the lower layer of crustose lichens lack hydrophobic materials, indicating a role in the uptake of water and solutes to the tissue. In fruticose lichens, the central core of stems may be hollow, and may have hyphae oriented in a woven pattern, and the hyphae may be thick-walled and multi-layered. The core may serve a number of functions; including strength and stability [39]. The matted anatomy of most lichens is particularly important for uptake and storage of water. Though water can be taken up rapidly, even from condensation at night, water is also lost. Thus the anatomy is closely linked to the functioning of the thallus. Water is necessary for metabolic processes, and in the absence of water, the lichen slows or stops its metabolic processes [25]

Lichens as Bioindicators

Lichens are increasingly being used as air quality biomonitors [17] because they have several advantages over electronic monitors. Which are expensive and their use and maintenance are not simple or cheap. They are limited to a few elements or chemical compounds and have no intrinsic relationship with the biological effect of the contaminants [20]. By contrast, biomonitors are available for free and there are millions of them already functioning throughout the world [21]. They integrally reflect the environmental influence over organisms and can be understood and used by the common citizen with minimal training. [12].

There is a long history of using lichens as indicators of air pollution [1]; [2]; [3]; [4]; [5]; [6]; [7]. In tropical regions, poor knowledge of lichen taxonomy does not affect basic biomonitoring because, this method does not require species identifications [31]. Air biomonitoring is particularly developed in Europe [13], where the lichen *Hypogimnia physodes* is used as a standard species [21]. Lichens accumulate and tolerate metals to a high degree because of their relatively large surface area, and slow growth rate. Because of the lack of cuticle and epidermis, and their poikilohydric nature, accumulation of air borne metals occurs by particle trapping [23], active uptake of anions, passive adsorption of cations and ion exchange [30].

Generally, there are three categories of lichens -one group of lichen disappear when the pollution starts, the second group are resistance to pollution, and the third group appears when pollution begins [15]. Most of the fruticose lichens are sensitive [16] where as foliose and crustose lichens such as *Cladonia convoluta, C. rangiformis, Neophuscelia pulla, Xanthoparmelia taractica, Xanthoria* sp. etc are resistant species and have also been reported as indicator of copper mining areas in Northern

Greece [15]. For decades, lichens have been known as good bio-accumulator for heavy metals and other inorganic air pollutants [12]; [13]; [14]; [15]; [16]. Application of biomonitors is the one of the suitable method to monitor these metals in Kathamndu, as some crustose and foliose lichens are available in and around Kathmandu [7]. There is very close statistical relationship between the accumulated heavy metal contents in lichens and the heavy metal pollution measured in air [25]; [26]. For example, a remarkable correlation was found between the deposition values and the corresponding accumulation values of exposed samples of *Hypogymnia physodes* in an emission related examination around a Danish steel works [37]. It is now well known that the production of geothermal energy may affect the surrounding environment. Excluding geological and geophysical effects, the environmental impact is related to the emission in to the atmosphere of significant amounts of uncondensable greenhouse gases, as well as elements and compounds of toxicological relevance which may be dangerous to public health [42]; [43]. They can be used as sensitive indicators to estimate the biological effects of pollutants by recording changes at the community or population level, and as accumulative monitors of persistent pollutants, which can be assessed by assaying their trace element content [11]. The use of lichens as biomonitors of geothermal air pollution from a physiological perspective, there are three specific areas where there is a considerable amount of information available; on the patterns of species distribution in relation to contrasting substrates that have differing ionic environments, on metal ion uptake and on sulphur dioxide substrate interactions.

[9] classified the origin of elements found in lichen thalli as two-fold; particulate atmospheric fallout and ionic solutions drops, the latter delivered as rainfall or as surface runoff. [10] indicated a large range in the elemental uptake of lichens that varied according to elemental characteristics of the substrate and environmental factors. The response of lichens to air pollutants is well understood. No one has suggested that lichens are not useful for indicating the effects of air pollutants, to the contrary that, the lichens are reliable indicators of change in emissions effects. Lichen response to pollutants has been questioned, the field is not without challenges to the basic concepts, and most of these are based on a careful assessment. For example, [23] rejected the concept of lichen species response to sulphur dioxide, and proposed that, drought was responsible for major declines in lichen species diversity. However, this was debunked by [25], but [27] went on to show that, drought and other effects could interfere with a species response to sulphur dioxide.

Air pollution effects theories have been challenged in other venues as well. For example, the concept of forest decline in Europe and North America [29] was challenged and found wanting [29]; [30]. The most problematic issue facing bio-monitoring currently is the shift in pollutant character from acidity due to sulphurous compounds to that from nitrogenous compounds. Lichen biomonitoring and bioindication has striven to make lichen bioindication relational to the response of other ecosystem components. For example, [8] correlated lichen zonation with soil sulphates concentration around an iron sintering plant at Wawa, Ontario. Their zones of damage corresponded with damage zones to vegetation extending 32 kms from the plant as defined by [21], including areas where damage to tips of emergence tree crowns were slightly injured by sulphur dioxide.

The consistency of the relationship between sulphur dioxide, lichen response and vascular plants was critically assessed and accepted by [19]. Due to the concern surrounding air pollution effects in the early 1980's, [17] recognized the mistrust on the part of non-biologists toward bio-indication and bio-monitoring with lichens, but provided ample evidence that lichen bio-indication and bio-monitoring has been accepted and developed on a variety of scales from local to national [28]; [29]. Since 1970 - 2000, the number of research dealing with lichen bio-indication has increased exponentially and has branched into respected and dedicated air quality journals.

Statement of the problem

Air pollution load is increasing day by day as result of human activities so the need to check mate the atmospheric environment is becoming necessary and there is lack of sufficiently sensitive and inexpensive techniques that permit the simultaneous analyses of many air contaminants [30]. The need of using sensitive lower organism lichens as bio-monitors is very necessary although, there are some groups of lichens that become less conspicuous when pollution becomes so persistent [34].

Justification

This research provides information on the presence of pollutants in the atmospheric environment using lichens as bio-monitors. This because Air pollution is something that we cannot really ignore now-a-days. This is evident from the moment we step out of our house and are greeted with black coloured smog that hits us directly reminding us that breathing clean air is more of a distant dream. The majority of these come from automotive engines and industries. Since air pollution cause damages to the vegetation and materials on earth apart from damaging the human and animal health, so a high degree of air pollution control is essential [8].

Lichens have been used, accepted, and developed for monitoring of air pollution effects earlier than most other plant groups [37]; [38]; [39]. Application of lichens as biomonitors of air quality is one of the suitable methods to monitor heavy metals in cities [40]. Biomonitoring studies provide valuable information about the quantity and quality of pollutants in the atmosphere and can be very effective as an early warning system to detect environmental changes [9].

MATERIALS AND METHOD

Locations and description of the study areas.

Selected Areas in Kaduna State

The following areas were chosen for the study

1. Rido village (7º25″8.434″E, 10º24″43.224″N) is located in Chikun Local Government area, Kaduna state.
2. PZ, Zaria (7º43' 16.395″E, 11º6' 9.986N) is located in Sabon Gari Local Government Area, Kaduna State.
3. Buruku village (6º56' 53.399″E, 10º 41' 8.463″E) village is located at Kaduna – Birnin Gwari road and is also under Chikun local Government area, Kaduna state.
4. Botanical Garden, Ahmadu Bello University, Zaria (7º 37' 56.239″E, 11º 9' 52.169″N) is also under Sabon Gari Local Government area, Kaduna state. (figure 1) (GPS model 010 – 01534 – 01)

Criteria for selection of study areas

Rido village: This was chosen due to its proximity to the Kaduna Refining and Petrochemical Company (KRPC) and there is high traffic of heavy trucks conveying petroleum products, a highly polluted area (HPA),

Buruku village: This is local area with significant amount of heavy truck shuttling from northern to south western part of Nigeria and its proximity to dumping site. Considered to be least pollution area (LPA).

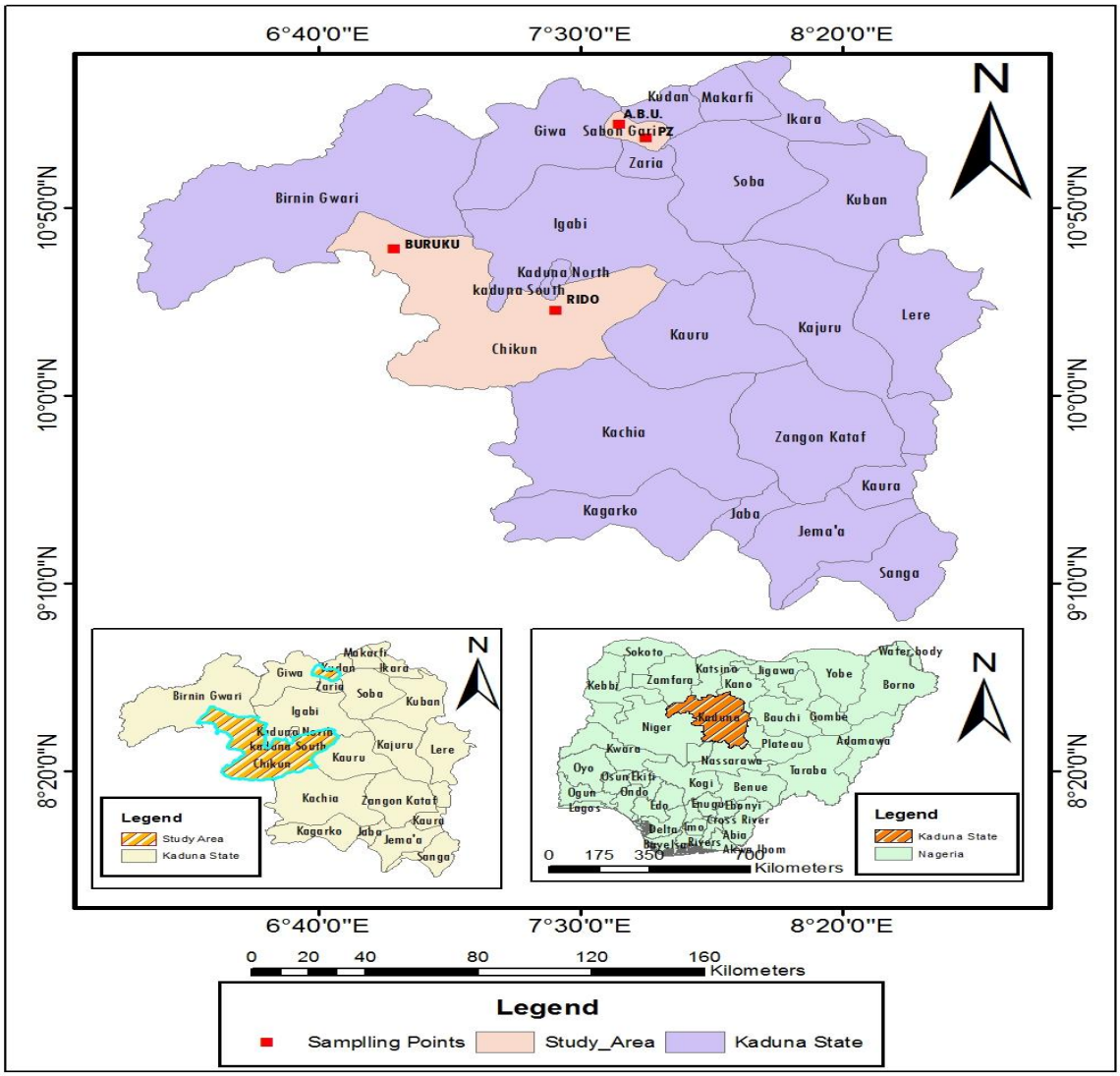

Figure 1; Map of Kaduna state showing the study areas

PZ areas: This is commercial area with higher traffic density, is considered to be High pollution area (HPA)

Botanical garden, Ahmadu Bello University was used as a control, Botanical garden can be said to be pollution free and naturally conserved, with little anthropogenic human activities, except for educational purposes, this is Considered to be Non pollution area (NPA)

Sample collection

Lichen distribution is directly influenced by substrate nature, moisture content and sunlight availability. All available substratum and habitat at each reference site were carefully examined, sufficient amount of lichen species were removed directly from the substrate. Lichen sampling was carried out during wet season (three months ie; July, August and September 2014) and dry season (three months ie; January, February and March 2015). For each month lichen sample was collected from three sites in the location selected. The lichen samples were collected within the range of 2 to 8m high from the ground of the trees. And the samples were collected in plastic bags and taken to the laboratory for further analysis, such as identification of samples and determination of target pollutants. Samples were taken to Herbarium in the Department of Biological Sciences Ahmadu Bello University Zaria for identification. The lichens was identify using 'key to nature' Natural history museum [43] with the help of expert in Taxonomy.

Location of the study site

Sites selected in each area were located up to 300 meters from main roads and densely traffic areas, and were more widely located (ie, above 300 meters) in industrial areas like Kaduna Refining and Petrochemical Company (KRPC). This is to avoid collection of samples from areas suspected to be of low deposition of heavy metals, and also to avoid collection of samples from areas of pollution sources which will give no desire result. Three sampling sites were selected at each sample location from which collection was made.

Sample preparation

The collected lichens samples were prepared according to [21] for metal analysis. Lichen samples were thoroughly cleaned, followed by washing the external surface in running distilled water. Representative samples for each sampling site were washed with deionized water and shade dried for two weeks. The dried lichens were then ground in a hand mill (using mortar and pestle) to a uniform size by sieving through a 2 μm sieve.

Determination of NO_3

Determination of Nitrogen (v) oxides (NO_3) was carried out at Soil Science Laboratory, Department of Soil Science, Ahmadu Bello University, Zaria. Phenoldisulphate acid method (APHA, 2005) was used to measure nitrate-nitrogen in the sample. About 50mls of the sample was evaporated to dryness and 1ml of Phenoldisulphate acid added to the residue. This was left to stand for 10minutes, before 10ml of distilled water was added to it until the residue was completely dissolved. Then 5ml of 0.12N potassium hydroxide was added to the solution, this was again diluted with distilled water to 50ml, and a yellow colour was formed. the absorbance of the yellow coloured was measured using Hach Spectrophotometer model DR/890 at 493.

Determination of SO_2

Determination of Sulphur dioxides (SO_2) was carried out at Soil Science Laboratory, Department of Soil Science, Ahmadu Bello University, Zaria. According to [15] a mass of 1g of each lichen sample was weighed into a flask and 21 ml of 6:1 mixture of concentrated Hydrogen trioxonitrate (HNO_3) and Hydrochloric acid (HCl) was added. The mixture was gently heated to 80°C and then the temperature was raised to 150°C to achieve complete dissolution. Sulphur dioxide (SO_2) was determined according to (APHA, 2005) at 420nm using Bosch and Lomb Spectronic -70.

Statistical analysis

The Mean values of the pollutants such as heavy metals, NO_3 and SO_2 at the period of sampling for all locations and for all lichens species were calculated. Seasonal variability in the values of the pollutants was determined using t-test. Data obtained was analyzed statistically using analysis of variance (ANOVA) to test the differences in individual concentration in each species found in the different locations. Duncan's multiple range test (DMRT) was used to separate the means where there is significant differences. Statistical Package for the Social Science (SPSS) version 19.0.0.247 (2016).

RESULT

Lichens species diversity in the study areas

Five lichens species were found; the most common species were foliose and fruticose forms. Tree Trunks contain the large number of foliose species.(see plates 1-5) in Appendix I

i, *Dictyonemma glabratum;* a fruticose lichen is formed by the symbiosis between cyanobacteria and basidiomycete fungi. It is also known as Basidiolichens. It was collected from ABU Botanical garden, during dry season.

ii, *Flavoparmelia caperata:* a foliose lichen was collected from Rido during the dry and wet seasons and Buruku during wet season. Medium to large foliose lichen that has a very distinctive pale yellow green upper cortex when dry. The rounded lobes, measuring 3–8 mm (0.1–0.3 in).

iii, *Physcia* spp: also foliose lichen. This was discovered and collected from PZ area and Buruku village during dry season.

iv, *Phaeophyscia sp* (Neck.) Moberg: this was collected from PZ area during wet season. Presence of closely pressed narrow radiating lobes, variable in colour (grey, green-grey to brown, green when wet). Widespread on nutrient-

enriched bark and other surfaces, tolerant of Nitrogen pollution they are foliose that normally colonize rough bark of the old trees. Thallus foliose, often forming rounded colonies with evenly radiating lobes, light to dark or brownish grey, green when wet, soralia rounded and convex, mostly laminar, but narrower and marginal towards the periphery of the colony, lower medulla white, orange pigment sometimes developing in the soralia and on damaged areas of cortex, apothecia infrequent, with rather thick, smooth margins and dark discs. Widespread and common on bark, walls and concrete.

V. *Xanthopermelia spp:* this was found in and around the ABU Botanical garden during wet season. The summary of all these are shown in (Table 1).

TABLE 1: LICHENS SPECIES FOUND IN FOUR LOCATIONS IN KADUNA STATE.

LICHENS SPECIES					
LOCATIONS	**DESCRIPTION**	**WET SEASON**	**DESCRIPTION**	**DRY SEASON**	**DESCRIPTION**
PZ	HPA	*Phaeophyscia spp*	Foliose	*Physcia spp*	Foliose
ABU	NPA	*Xanthoparmelia spp*	Foliose	*Dyctyonema glabratum*	Fruticose
Rido	CPA	*Flavoparmelia capirata*	Foliose	*Flavoparmelia capirat*	Foliose
Buruku	LPA	*Flavoparmelia* capirata	Foliose	*Physcia species*	Foliose

Keys: HPA: High pollution area, CPA: Controlled pollution area, LPA: Least pollution area, NPA: Non pollution area

NO_3 and SO_2 pollution load in lichens from sampled areas

The concentration of NO_3 ranged from 0.051 – 0.140mg/L which was recorded in *Xanthoparmelia caperata* and *Flavoparmelia caperata*. And SO_2 concentration ranged from 62.892 – 238.989mg/L the content recorded from *Xanthoparmelia* spp and *Physcia* spp collected from Buruku which also reported with highly significant different at P≤0.05 respectively (Table 2). The mean concentration of SO_2 and NO_3 also like heavy metals is determined in all locations and seasons. Values are expressed as means ± SEM (standard error of mean), NO_3 ranged from 0.091 – 0.102mg/L, SO_2 values ranged from 72.954 – 496.845mg/L, the values of the locations are shown in (Figure 7 and 8) as PZ area is significantly higher in SO_2 concentration while Rido is significantly lower in NO_3 concentration respectively. The mean monthly concentration of SO_2 and NO_3 shows that NO_3 ranged from 0.075 – 0.125mg/L, SO_2 ranged from

133.959 – 786.791mg/L (Table 3). Table 4; shows the mean concentration of NO_3 and SO_2 at PZ area, NO_3 ranges from 0.062 – 0.158mg/L, and SO_2 ranged from 45.285 – 260mg/L. NO_3 were recorded higher in January with 0.0.158±0.018 and SO_2 recorded higher in July with 260.780±150.220mg/L. ABU Botanical garden the mean concentration of NO_3 and SO_2. NO_3 ranged from 0.053 – 0.185mg/L, SO_2 content ranged 37.735 – 105.655mg/L. NO_3 and SO_2 were recorded higher in July with 0.185±0.044 and 105.655±15.095mg/L see (Table 5). The Rido village as shown in (Table 6), the mean concentration of NO_3 and $SO_{2,}$ the content of NO_3 ranged from 0.040 – 0.184mg/L, SO_2 content ranged from 188.675 – 303.765mg/L. NO3 and SO_2 are recorded higher in January and with 0.184±0.114 and 303.765±7.545 The mean concentration of NO_3 and SO_2 in Buruku, Show that the values of NO_3 ranged from 0.071 – 0.132ppm and SO_2 values ranged from 196.220 – 218.860mg/L, with highest content recorded in the month of March and July. NO_3 and SO_2 were recorded in March with 0.132±0.027 and 218.860±7.550mg/L (Table 7). The mean seasonal variation of NO_3 and SO_2 show that the value of NO_3 ranged from 0.096 – 0.100mg/L, SO_2 ranged from 140.505 – 355.343mg/L (Table 8).

TABLE 2: MEAN CONCENTRATION OF SO_2 AND NO_3 IN LICHENS SAMPLE FROM FOUR LOCATIONS IN KADUNA STATE

Lichens	Locations	NO_3 (mg/L)	SO2(mg/L)	Mean
Physcia spp.	PZ area	0.094 ± 0.012^{ab}	121.988 ± 59.553^{bc}	61.04 ±29.78
Phaeophyscia spp.	PZ area	0.111 ± 0.017^{ab}	90.532 ± 14.051^{bc}	45.32 ± 7.03
Dictyonema Glabratum	ABU	0.129 ± 0.027^{a}	83.015 ± 10.125^{bc}	41.57 ± 5.08
Xanthoparmelia caperata	ABU	0.051 ± 0.005^{b}	62.892 ± 15.301^{c}	31.47 ± 7.65
Flavoparmelia caperata	Rido	0.126 ± 0.038^{a}	158.498 ± 29.873^{ab}	79.31 ± 14.96
Flavoparmelia caperata	Rido	0.094 ± 0.015^{ab}	216.359 ± 10.064^{a}	108.23 ± 5.04
Physcia spp.	Buruku	0.079 ± 0.007^{ab}	238.989 ± 10.614^{a}	119.53 ± 5.31
Flavoparmelia caperata	Buruku	0.140 ± 0.036^{a}	206.288 ± 5.031^{a}	103.21 ± 2.53
Mean		0.10 ± 0.02	147.32 ± 18.69	
P-value		0.144^{ns}	0.000**	

Note: Value are express as mean ± SE (Standard error) significant different area taken at p≤0.05 while non significant different are taken at p>0.05, **highly significant, ^{ns} = not significant

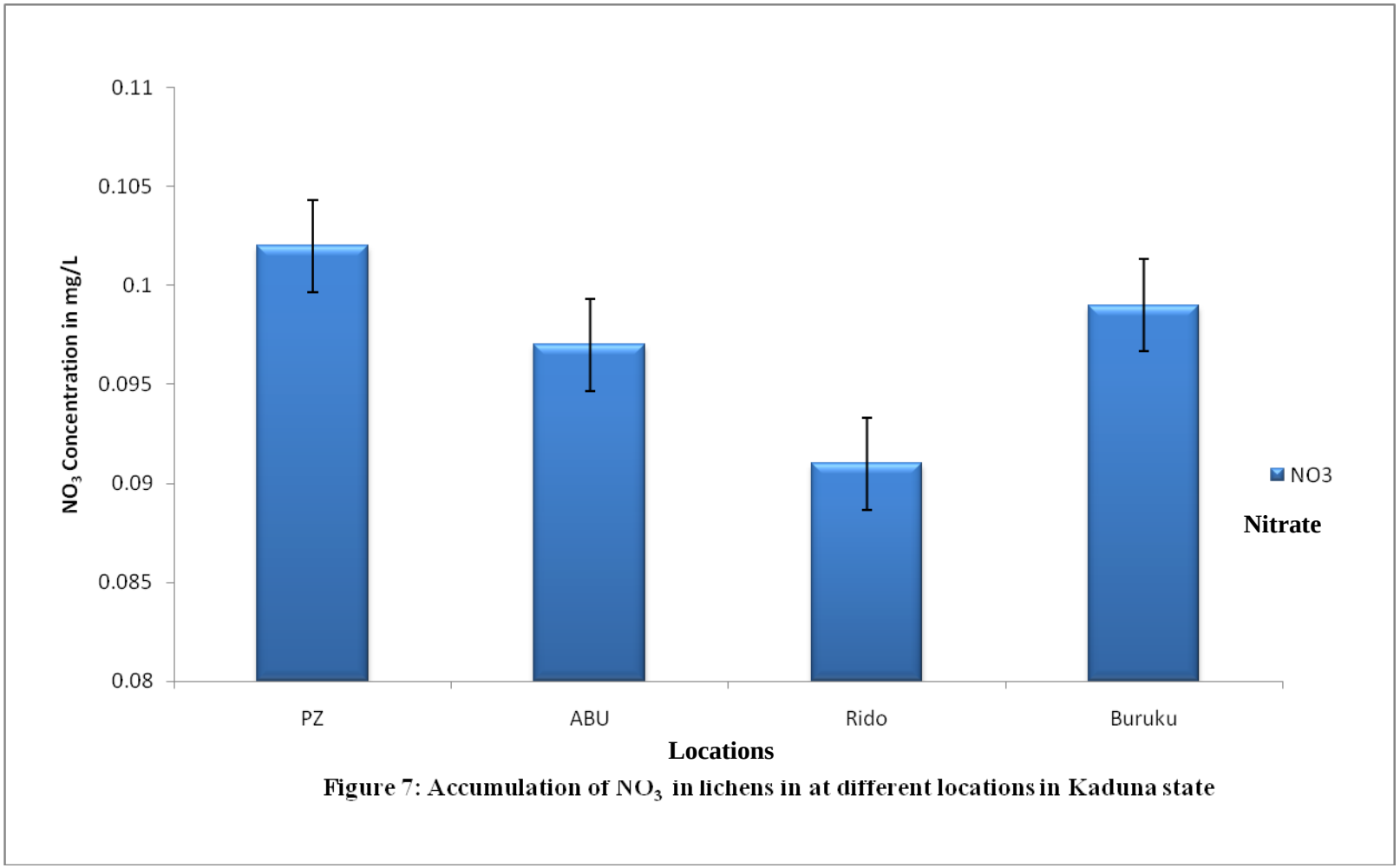

Figure 7: Accumulation of NO_3 in lichens in at different locations in Kaduna state

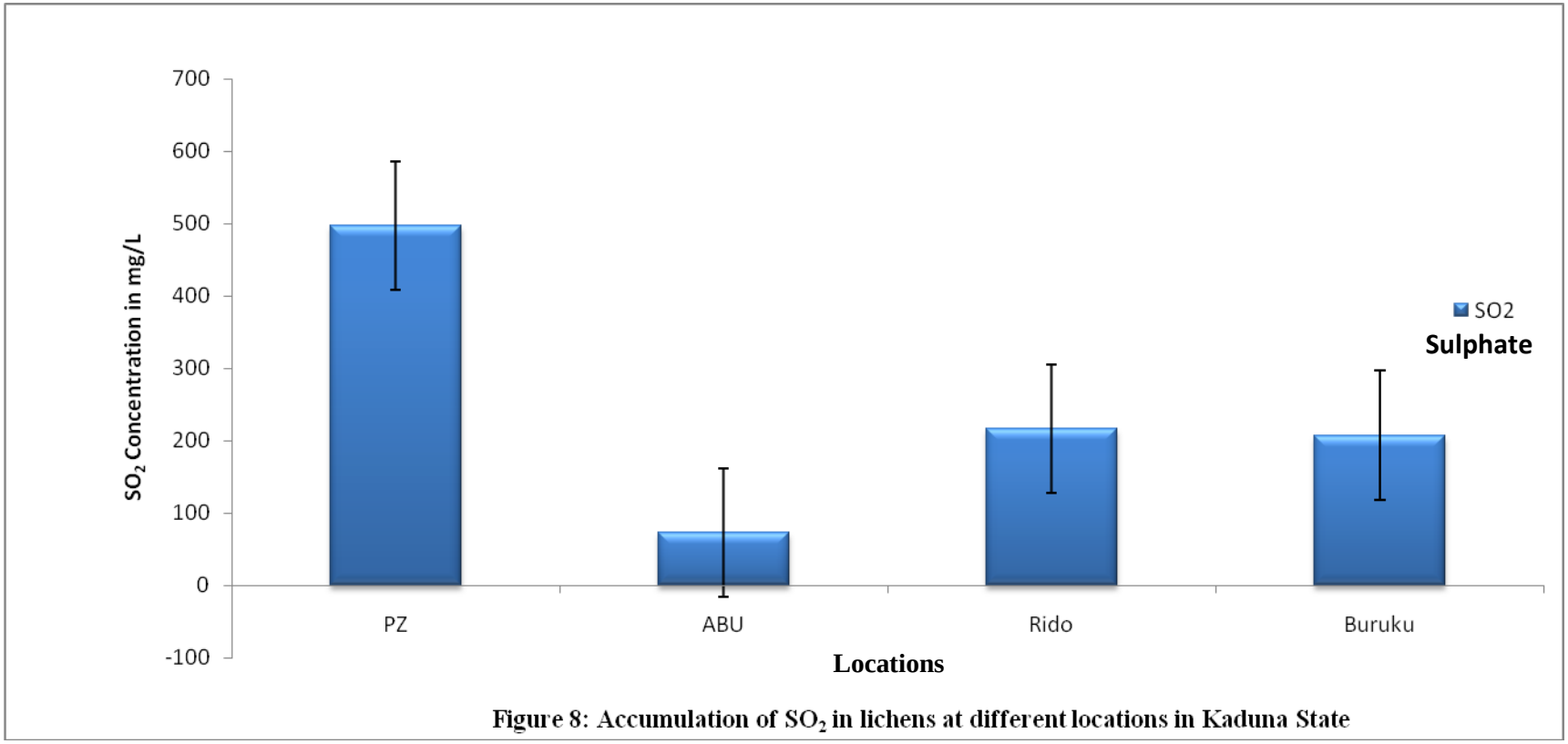

Figure 8: Accumulation of SO_2 in lichens at different locations in Kaduna State

Table 3: MEAN CONCENTRATION OF NO_3 AND SO_2 IN LICHENS SAMPLE ACROSS THE MONTHS IN SABON-GARI AND CHIKUN LOCAL GOVT AREA OF KADUNA STATE

Months	NO_3 (mg/L)	SO_2 (mg/L)
January	0.125 ± 0.027^a	141.504 ± 24.025^a
February	0.075 ± 0.008^a	133.959 ± 28.587^a
March	0.090 ± 0.013^a	149.051 ± 31.691^a
July	0.119 ± 0.022^a	786.791 ± 492.992^a
August	0.092 ± 0.015^a	133.959 ± 25.583^a
September	0.086 ± 0.011^a	145.279 ± 30.043^a
Mean	0.09 ± 0.02	248.42 ± 31.65
P-value	0.32^{ns}	0.16^{ns}

Note: Values are expressed as means ± SE (standard error), significant different are taken at p-value ≤0.05. Non-significant difference is taken at p-value >0.05, ^{ns} = not significant

Table 4: MEAN CONCENTRATION OF NO_3 AND SO_2 IN LICHENS SAMPLE FROM PZ AREA

Months	NO_3 (mg/L)	SO_2 (mg/L)
January	0.158 ± 0.018^{a}	113.200 ± 22.640^{a}
February	0.088 ± 0.018^{bc}	90.565 ± 30.185^{a}
March	0.088 ± 0.018^{bc}	67.920 ± 22.640^{a}
July	0.123 ± 0.018^{ab}	260.780 ± 154.220^{a}
August	0.097 ± 0.009^{bc}	60.375 ± 15.095^{a}
September	0.062 ± 0.009^{c}	45.285 ± 15.090^{a}
Mean	0.10 ± 0.02	106.35 ± 43.31
P- value	0.04^{*}	0.13^{ns}

Note: Values are expressed as means ± SEM (standard error of mean), means with different superscript down the columns are significantly different at p-value ≤0.05. Non-significant difference is taken at p-value >0.05, *significant, ^{ns} = not significant

Table 5: MEAN CONCENTRATION OF NO_3 AND SO_2 IN LICHENS SAMPLED FROM ABU BOTANICAL GARDEN

Months	NO_3 (mg/L)	SO_2 (mg/L)
January	0.079 ± 0.009^a	52.830 ± 7.550^a
Februay	0.053 ± 0.000^a	37.735 ± 7.545^a
March	0.062 ± 0.009^a	67.925 ± 7.545^a
July	0.185 ± 0.044^a	105.655 ± 15.095^a
August	0.097 ± 0.062^a	75.470 ± 0.000^a
September	0.106 ± 0.018^a	98.110 ± 37.730^a
Mean	0.09 ± 0.02	72.95 ± 12.57
P-value	0.19^{ns}	0.18^{ns}

Note: Values are expressed as means ± SE (standard error), significant different are taken at p-value ≤0.05. Non-significant difference is taken at p-value >0.05, ^{ns} = not significant

Table 6: MEAN CONCENTRATION OF NO_3 AND SO_2 IN LICHENS SAMPLED RIDO VILLAGE

Months	NO_3 (mg/L)	SO_2 (mg/L)
January	0.184 ± 0.114[a]	303.765 ± 7.545[a]
February	0.071 ± 0.018[a]	211.315 ± 30.185[a]
March	0.079 ± 0.026[a]	241.500±30.190[a]
July	0.040 ± 0.005[a]	218.870±22.650[a]
August	0.097 ± 0.044[a]	188.675 ± 7.545[a]
September	0.079 ± 0.044[a]	233.955 ± 7.545[a]
Mean	0.09 ± 0.04	192.76 ± 17.61
P-value	0.58[ns]	0.53[ns]

Note: Values are expressed as means ± SE (standard error), significant different are taken at p-value ≤0.05. Non-significant difference is taken at p-value >0.05, [ns] = not significant

Table 7: MEAN CONCENTRATION OF NO_3 AND SO_2 IN LICHENS SAMPLE FROM BURUKU VILLAGE

Months	NO_3 (mg/L)	SO_2 (mg/L)
January	0.071 ± 0.018^a	196.220 ± 0.000^a
February	0.088 ± 0.018^a	196.220 ± 0.000^a
March	0.132 ± 0.027^a	218.860 ± 7.550^a
July	0.132 ± 0.009^a	218.860 ± 7.550^a
August	0.079 ± 0.009^a	211.315 ± 15.095^a
September	0.097 ± 0.009^a	203.765 ± 7.545^a
Mean	0.09 ± 0.01	207.54 ± 6.29
P- value	0.13^{ns}	0.28^{ns}

Note: Values are expressed as means ± SE (standard error), significant different are taken at p-value ≤0.05. Non-significant difference is taken at p-value >0.05, ^{ns} = not significant

Table 8: MEAN SEASONAL VARIATION OF NO_3 AND SO_2 CONCENTRATION

	Pollutants	
Seasons	**NO_3 (mg/L)**	**SO_2 (mg/L)**
Dry	**0.099 ± 0.010**	**355.343 ± 169.888**
Wet	**0.096 ± 0.011**	**141.505 ± 15.652**
Mean	**0.09 ± 0.01**	**248.42 ± 92.77**
P – value	**0.82^{ns}**	**0.22^{ns}**

Note: Values are expressed as means ± SEM (standard error of mean), Significant difference are taken at p≤0.05 while non-significant differences between wet and dry seasons are taken at p-value >0.05, ^{ns} = not significant

DISCUSSION

Dictyonema glabratum found at ABU Botanical garden during dry season, a fruticose was formed by the symbiosis between algae/Cyanobacteria and Basidiomycete fungi it is also known as Basidiolichens, and also known as macrolichens, it is common and widely available lichen species and considered to be ecologically important to Nigeria as it is one among many lichens that fix atmospheric Nitrogen into the Soil, which makes them (the lichens) natural fertilizers. *Dictyonema glabratum* grows in curly masses around objects, such as tree trunks [1].

Flavopermelia capirata: The lobes of the thallus of this species may be smooth, but quite often have a wrinkled appearance especially in older specimens. Widespread on well-lit acid-barked trees, wood and other surfaces, now colonising urban and other disturbed sites, previously affected by acid rain.

Physcia species: The name of lichen originated from the Greek *physckέ*, used to describe the large intestine, a sausage or a blister and referring to thalli with hollow lobes. *Physcia* is a cosmopolitan genus of 75 species. It is distinguished from most other foliose Physciaceae mainly by its pseudoparenchymatous upper cortex in combination with atranorin as a cortical substance [3]. *Physcia* species was also found by [9] in Nigerian guinea Savannah which coincides with areas where this research was conducted.

Phaeophyscia species: This was collected during wet season at PZ area. All the lichens collected are foliose and fruticose species which are mainly found on old tree trunk with rough bark, because roughness increases humidity which is the factor contributing toward the survival of the lichens, This is according to [11] that, humidity caused by bark roughness may be an important factor in establishing different species that attach themselves more easily to irregular surfaces, but less to smooth surfaces.

The genus *Xanthoparmelia* (Vain.) Hale, comprised of approximately 750 species, constitutes a major part of the family [16]. All *Xanthoparmelia* species share key taxonomic characters, including the degree of attachment to the substrate, colour of the lower surface (pale brown to ebony black), presence of isidia of different types (cylindrical to globose), shape of the lobes and the medullar chemistry [18]; [19]. The history of *Xanthoparmelia* as an individual genus has been relatively brief, as all *Xanthoparmelia* species were only separated from *Parmelia* as recently as 1974, and few publications on this genus even existed up until 1959. Only since 1964 have virtually all of the 300 or so additional new species of *Xanthoparmelia* been described, with a total of 750 species reported [22]; [23]; [24].

Lichens have a higher capacity to accumulate and store heavy metals for a long time because of their morphological and ecological peculiarities. Lichens are widely used as plant material to investigate or biomonitor airborne heavy metals [31].

The NO_3 and SO_2 background content was reported high in PZ area while lowest was reported in Rido with 0.102±0.010 and 0.09±0.021mg/L respectively. The Nitrogen found in thalli was however highly linked to moist Nitrogen deposits, but was also correlated with NO_3 present in air as well as in the lichen species [12]. SO_2 content was also reported higher in PZ area with 496.854±342.143mg/L while the lowest value was recorded in ABU Botanical garden with value of 72.954±8.877mg/L the result of measured atmospheric SO_2 showed the highest pollution at the PZ area since SO_2 can be transported far away from its emission site. It is true to say that this gaseous phototoxic pollutant is one of the most important factors which have negative effect in epiphytic vegetation even at remote site [1]. According to [39] high level of SO_2 and NO_3 can cause a reduction of pH of lichen to this respect, it shall be highlighted that atmospheric pollution of this kind has lead to the less abundance of some lichen species like *Lorama pulmonaria* and *Ramalina farinacea.* So with this the scarce abundance of lichen in PZ area is attributed to the higher level of this pollution. According to [7], the number of pollutants intolerant of lichens species drops from 10 to 5 with an associated decline in species number

when the wet and dry seasons NO_2 concentrations are greater than 0.46 ppb and 0.15 ppb (respectively the median wet and dry seasons pollutants concentrations observed in this study). The presence of the intermediate tolerant lichen species when the NO_2 is above 0.46 ppb is roughly unchanged but the species number is lower than when the NO_2 concentration is below 0.46 ppb [19]. Two of the pollutant tolerant species of lichen (*Phaeophyscia rubropulchra* and *Pyxine sorediata*) are absent from the sites that experience NO_2 concentrations above 0.46 ppb. However, two pollution tolerant species (*Parmelia sulcata* and *Melanelia subaurifera*) appear not to be impacted by NO_2 concentrations above 0.46 ppb. On the other hand, it is possible to differentiate the effect of traffic released pollutants on the different studied sites, which helps raise the issue of the need to carry out better controls on the quality of the air in our country, as well as to control the level of pollutant emissions in the vehicles circulating [26].

The mean monthly variation of heavy metal, SO_2 and NO_3 accumulation. Showed that in January heavy metal accumulation of Mn was the highest 1.799±0.297 Mn>Cr>Pb>Zn>Cd, while SO_2 high than NO_3 and NO_3 high content is recorded in January and lowest in February. With this the accumulation of heavy metals in lichens or any plants specimens can attain higher or lower contents in respective of seasonal variability. And the morphology of lichens and mosses does not vary with seasons; thus accumulation can occur throughout the year. Lichens and mosses usually have considerable longevity, which led to their use as long-term integrators of atmospheric deposition [43].

CONCLUSSION

Lichens species found in all locations were; *Dictyonema glabratum, Flavomarmelia caperata, Phaeophyscia* spp*, Xanthoparmelia caperata* and *Physcia* spp. There was significant difference among the lichens for all the pollutants studied. *Flavomarmelia caperata* have the higher accumulation of the most pollutants with the mean value of (97.39 ± 7.64ppm). The highest concentration of pollutants was recorded in PZ area with the mean value of (248.96 ± 6.08ppm). ABU Botanical garden had the lowest content of most of the pollutants with mean value of (36.94 ± 2.26ppm), SO_2 has the highest deposition in lichens in all the locations. This work has provided an insight into the geospatial and seasonal variation of pollutants in all the study areas, and an area whose air quality status has not previously been studied like Buruku. The study has shown that anthropogenic sources such as metals work shop, agro-chemicals fossil combustion etc) of air pollution appear to be the predominant influence on NO_3 concentrations and potentially also on the lichen species diversity in most of the study areas, especially in the PZ area. The study has shown that monitoring programs such as a lichen desert and lichen forest can reveal the locations where ecological detriment is occurring that maybe associated with air pollution even at low levels. The improved sensitivity to this passive biomonitoring methodology applied here will also be applicable to other monitoring studies of ambient pollutants.

RECOMMENDATION

The continuous study of lichens species is recommended considering their significance in determining air quality in our environment. The use of epiphytic lichens biomonitoring particularly; *Dictyonema glabratum*, *Flavopermelia capirata, Xanthoparmelia* species, *Physcia* species and *Phaeophyscia* species is recommended because it provides a cost–effective approach for monitoring of atmospheric pollutants. It also recommended continuous monitoring and investigating the pollutants, as it is being done in some countries, considering the toxicological effect they posed to human health and environment.

CONTRIBUTION TO KNOWLEDGE

1. Establishing the presence of the common lichen species such as; *Dictyonema glabratum,*(Fruticose lichen) *Flavopermelia carperata, Xanthopermelia caperata*, *Phaeophyscia* species and *Physcia*

species (Foliose lichen) in the study area.

2. Quantifying the relative concentration of the pollutants adsorbed by lichens;

i. NO_3 and SO_2 were recorded as (0.10 ± 0.02mg/L) and (147.32 ± 18.69mg/L) respectively, where as SO_2 is the highest pollutants absorbed by lichens.

ii. *Flavoparmelia caperata* trapped highest content of pollutant with (97.39 ± 7.64ppm), *physcia* specie (90.74 ± 17.63ppm), *Phaeophyscia* specie (45.80 ± 7.36ppm), *Dictyonema glabratum* (41.84 ± 5.12pppm), and *Xanthoparmelia caperata*, (32.02 ± 7.73ppm).

iii. PZ area had the highest content of pollutants with (248.96 ± 6.08ppm) which make it the highly polluted area, Rido with (108.51 ± 2.07ppm), Buruku with (104.53 ± 1.95ppm), and ABU Botanical garden with (36.94 ± 2.26ppm).

REFERENCE

1. Abdullahi, M. Z., Saat, A. B. and Hamzah, Z. B. (2012). Assessment of the impact of petroleum and petrochemical industries to the surrounding areas in Malaysia usingmosses as bioindicator supported by multivariate analysis. *Journal of Environmental Monitoring and Assessment*, 184 (6): 3959-3969.
2. Adamo, P., Crisafulli, P., Giordano, S., Minganti, V., Modenesi, P., Monaci, F., Pittao, E., Tretiach, M. and Bargagli, R (2007). Lichen and moss bags as monitoring devices in urban areas. Part II: Trace element content in living and dead biomonitors and comparison with synthetic materials. *Environmental Pollution.* 146: 392-399.
3. Adamo, P., Giordano, S., Vingiani, R., Castaldo C. and Violante, P. (2003). Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy). *Environmental Pollution*, 122: 91-103.
4. Ahmadjian, V., (1990). What have synthetic lichens told us about real lichens? *Bibliothecha Lichenology,* 38: 3–12.
5. Aksoy, A., Leblebici, Z. and Halici, M. G. (2010). Biomonitoring of Heavy Metal Pollution Using Lichen (*Pseudevernia furfuracea* (L.) Zopf.) Exposed in Bags in a Semi-arid Region, Turkey. In: Ashraf, M., Ozturk, M. and Ahmad, M. S. (Eds.). *Plant Adaptation and Phytoremediation*, Springer, Netherlands. pp. 59-70.
6. Alimonti, A., Francesco, P., Michael, K. Beatrice, B. and Sergio, C. (2000). Reference value for Chromium, Nickel and Vanadium in urine of youngsters from the urban area of Rome. *Journal of Environmental Monitoring*, 2: 351 – 354.
7. Allen, S. E., (1989). Chemical analysis of ecological materials. (Second Edition). *Blackwell Scientific Publications,* Oxford: 368pp.
8. American Public Health Association (APHA) (2005). Standard method for the examination of water and waste water. (second edition). *American Public Health Association* Inc., New York. 1193pp.
9. Anderson, A., Haovmand, M. F. and Jornsen, I., (1978). Atmospheric heavy metal deposition in the Copenhagen area. *Journal of Environmental Pollution,* 17: 401- 406.
10. Aniefiok, E. I., Imaobong, I. U., Udousoro and Udo, J. I. (2014). Distribution of some atmospheric heavy metals in lichen and moss samples collected from Eket and Ibeno Local Government Areas of Akwa Ibom State, Nigeria. *American Journal of Environmental Protection.* 2 (1): 22-31.
11. Armannsson, H., and Kristmannsdóttir, H. (1992). Geothermal Environmental Impact. *Geothermics*, 21: 869-880.
12. Aslam, M., Verma, D. K., Dhakerya, R., Rais, S., Alam, M. and Ansari, F. A. (2011). A

comparative study on uptake and accumulation of heavy metals in some plant, *Journal of Environmental and Earth Science*, 4(12):1060–1070.

13. Axtman, R. C. (1975). Environmental impact of a geothermal power plant. *Journal of Environmental Science,* 187: 795-803.
14. Backor, M. and Loppi, S., (2009). Interactions of lichens with heavy metals. *Journal of Biologial Plantarum,* 53(2): 292-299.
15. Bajpai, R. and Upreti D. K. (2012). Accumulation and toxic effect of arsenic and other heavy metals in a contaminated area of West Bengal, India. In the lichen *Pyxine cocoes* (Sw.) Nyl. *Ecotoxicology and Environmental Safety*, 83: 63–70.
16. Bajpai, R., Upreti, D. K. and Mishra, S. K. (2004). Pollution monitoring with the help of lichen transplant technique at some residential sites of Lucknow. *Journal of Environmental Biology*, 25: 191-195.
17. Bako, S. P., Afolabi, S. and Funtua, I. I. (2008). Spatial distribution and heavy metal content of some bryophytes and lichens in relation to air pollution in Nigeria's Guinea Savanna. *Journal of Environment and Pollution*, 33 (2): 195-206.
18. Balaguer, L. and Manrique, E. (1991). Interaction between Sulphur dioxide and Nitrate in Some Lichens. *Journal of Environmental and Experimental Botany*, 31(2): 223-227.
19. Kimbrough, D. E., Cohen, Y. and Winer, A. (1999). A critical Assessment of chromium in the environment. *A Critical Review Environmental Science and Technology,* 29:1–46.
20. Klos, A., Rajfur, M., Sramek, I. and Waclawek, M. (2011). Use of Lichen and Moss in Assessment of Forest Contamination with Heavy Metals in Praded and Glacensis Euroregions (Poland and Czech Republic). *Journal of Water Air and Soil Pollution*, 222: 367-376.
21. Krahl-Urban, B., Papke, H .E., Peters, K. and Schimansky, C. (1988). Forest Decline: Cause-Effect Research in the United States of North America and Federal Republic of Germany. Published by the Assessment Group for Biology, Ecology and Energy of the Jülich Nuclear Research Centre for the U.S. *Environmental Protection Agency and German Ministry of Research and Technology.* 137 pp.
22. Kularatne, K. I. A. and Freitas, C. R. (2012), Epiphytic Lichens as biomonitors of airborne heavy metal pollution, *Journal of Environmental Botany,* 88: 24-32.
23. Moriarty, F. (1999). Ecotoxicology: *The Study of Pollutants in Ecosystem*, (Third Edition) Academic Press, London, 347pp.
24. Muir, P. S., and McCune, B. (1988). Lichens, tree growth, and foliar symptoms of air pollution: are the stories consistent? *Journal of Environmental Quality*, 17: 361-370.
25. Murphy, K. J., Alpert, P., and Cosentino, D. (1999). Local impacts of a rural coal burning generating station on lichen abundance in a New England forest. *Environmental Pollution.* 105 (3): 349-354.
26. Nash, T. H. (1989). Metal tolerance in lichens. In: Shaw, J. (Ed.). *Heavy metal tolerancm in plants*, evolutionary aspects. CRC Press, Boca Raton. Pp. 119-132
27. Nash, T. H., III and Egan, R. S. (1988). *The biology of lichens and bryophytes.* In: Nash, T. H. III and Wirth, V. (Eds.), Lichens, Bryophytes and Air Quality. *Journal Cramer,* Berlin-Stuttgart, pp. 11-22.
28. Nayaka, S., Upreti, D. K., Gadgil, M. and Pandey, V. (2003). Distribution pattern and heavy metal accumulation in lichens of Bangalore city with special reference to Lalbagh garden. *Current Science,* 84: 674-680.
29. Nayaka, S., Upreti, D. K., Punjani, B., Dubey, U. and Rawal. J. (2007). New records and notes on some interesting lichens of

family Roccellaceae from India. *Phytotaxonomy,* 10: 127-133.
30. Negi, H. R. (2003). On the patterns of abundance and diversity of macrolichens of Chopta-Tunganath in the Garhwal Himalaya. *Journal of Biosciences,* 25(4): 367–378.
31. Richardson, D. H.. Shore, S. M., Hartree, R. and Richardson, R. M. (1995). The use of Xray fluorescence spectrometry for the analysis of plants, especially Lichens, in Biological Monitoring. *The Science of the Total Environment*. 176: 97 – 105.
32. Riget, F., Asmund, G., and Aastrup, V. (2000). The use of lichen *Cetraria nivalis* and moss *Rhacomitrium lanuginosum* as monitors for atmospheric deposition in Greenland, *The Science of Toxicological and Environmental Chemistry,* 50: 157-166.
33. Rodrigo, A., Ávila, A. and Gómez-Bolea. A. (1999). Trace metal contents in *Parmelia caperata* (L.) A c h . Compared to bulk deposition, throughfall and leaf wash fluxes in two holms oak forests in Monsey (NE Spain). *Atmospheric Environment*. 33: 359-367.
34. Romic, M. and Romic, D. (2003). Heavy metal distribution in agricultural top soils in urban area. *Journal of Environmental Geology*, 43: 795- 805.
35. Ronen, R. and Galun, M., (1984). Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. *Journal Environmental and Experimental Botany.* 24: 239–245.
36. Uluozlu, O. D., Kinalioglu, K., Tuzen, M. and Soylak, M. (2007). Trace metal levels in lichen samples from roadsides in East Black Sea region, Turkey. *Journal of Biomedical and Environmental Sciences,* 20(3): 203-207,
37. Van Dobben, H. F. and Ter Braak, C. J. F. (1998). Ranking of epiphytic lichen sensitivity to air pollution using survey data: A comparison of indicator scales. *Lichenologist,* 31 (1): 27-39.
38. Van Dobben, H. F., Wlterbeek, H. T., Wamelink, G. W. and TerBrank, C. J. F., (2001). Relentionship between epiphytic lichens, trace elements and gaseous atmospheric pollutants. *Journal of Environmental Pollution* 112: 163-169.
39. Wastlhuber, R. and Loos, E., (1996). Differences between cultured and freshly isolated Cyanobiont from *Peltigera* sp is there symbiosis-specific regulation of a glucose carrier? *Lichenologist*, 28: 67–78.
40. WHO, (2000). Air Quality Guidelines for Europe, (2nd edition), Copenhagen, Denmark: *World Health Organization*, Regional Office for Europe, 273pp.
41. Yuan, X., Xiao, S., Taylor T. N. and Xiao, T. (2005). Lichen-like symbiosis 600 million years ago. *Science* 308 (5724): 1017–2000.
42. Zambrano, A. and Nash, T. H. III (2000). Lichen responses to short-term transplantation in Desierto de los Leones, Mexico City. *Environmental Pollution,* 107 (3): 407-412.
43. Zhu, F., Qu, L., Fan, W., Qiao, M., Hao, H. and Wang, X. (2011). Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environmental Monitoring and Assessment,* (4): 179 – 191.

APPENDIX I

Dictionema glabratum:
Body form: Fruticose
Habitat: Coticolous
Description: this lichens is formed by symbiosis between a basidiomycete fungus and cyanobacterium, making it both a basidiolichen and a cyanolichen, which is a very rare combination This makes *Dictyonema* more closely related to mushrooms than it is to most other lichens.

Xanthoparmelia caperata
Body form: Foliose
Habitat: Coticolous
Description: The genus *Xanthoparmelia* (Vain.) Hale, comprised of approximately 750 species, All *Xanthoparmelia* species share key taxonomic characters, including the degree of attachment to the substrate, color of the lower surface (pale brown to ebony black), presence of isidia of different types (cylindrical to globose)

Phaeophyscia spp
Body form: Foliose
Habitat: Coticolous
Description: Shelf-like filamentous, up to 7 cm across, composed of loosely interwoven but compacted, more or less horizontally arranged, erogenous fibrils bordered by a narrow, white margin. Thallus in section 0.5–1 mm thick, composed of an irregular photobiont layer and a thin medulla forming a white hypothallus; photobiont layer composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells;

Flavopermelia caperata
Body form: Foliose
Habitat: Coticolous
Description: Common greenshield lichen is a medium to large foliose lichen that has a very distinctive pale yellow green upper cortex when dry, usually have patches of granular soredia arising from pustules. The lobes of the thallus may be smooth, but quite often have a wrinkled appearance especially in older specimens. The lower surface is black except for a brown margin

Physcia spp*:* Body form: Foliose
Habitat: Courticours and some are Saxicolours
Description: Is a genus of lichenized fungi in the family Physciaceae. The genus name means "inflated" or "sausage-like. According to a 2008 estimate, the widespread genus contains 73 species.

APPENDIX II

Pb

Descriptives

HM

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.2610	.07422	.03030	.1831	.3389	.20	.39
2	6	.2798	.11511	.04700	.1590	.4006	.18	.50
3	6	.1785	.03136	.01280	.1456	.2114	.15	.24
4	6	.1768	.02365	.00966	.1520	.2017	.15	.22
5	6	.1317	.05839	.02384	.0704	.1929	.08	.24
6	6	.1342	.05900	.02409	.0723	.1961	.06	.21
7	6	.0975	.02261	.00923	.0738	.1212	.06	.12
8	6	.1422	.02382	.00972	.1172	.1672	.11	.16
Total	48	.1752	.08194	.01183	.1514	.1990	.06	.50

ANOVA

HM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.174	7	.025	7.041	.000
Within Groups	.141	40	.004		
Total	.316	47			

HM

Duncan

SPP	N	Subset for alpha = 0.05		
		1	2	3
7	6	.0975		
5	6	.1317	.1317	
6	6	.1342	.1342	
8	6	.1422	.1422	
4	6		.1768	
3	6		.1785	
1	6			.2610
2	6			.2798
Sig.		.244	.233	.586

Means for groups in homogeneous subsets are displayed.

Mn

Descriptives

HM

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	1.1993	.92161	.37625	.2322	2.1665	.62	2.98
2	6	1.4630	.85983	.35102	.5607	2.3653	.50	2.89
3	6	.6423	.43675	.17830	.1840	1.1007	.41	1.53
4	6	2.1165	.87676	.35794	1.1964	3.0366	.87	3.06
5	6	.8155	.49064	.20030	.3006	1.3304	.27	1.48
6	6	.8445	.42462	.17335	.3989	1.2901	.44	1.67
7	6	1.6898	.60902	.24863	1.0507	2.3290	.96	2.47
8	6	3.8378	1.61216	.65816	2.1460	5.5297	1.45	5.87
Total	48	1.5761	1.26374	.18240	1.2092	1.9431	.27	5.87

ANOVA

HM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.365	7	6.481	8.730	.000
Within Groups	29.696	40	.742		
Total	75.061	47			

HM

Duncan

SPP	N	Subset for alpha = 0.05		
		1	2	3
3	6	.6423		
5	6	.8155		
6	6	.8445		
1	6	1.1993	1.1993	
2	6	1.4630	1.4630	
7	6	1.6898	1.6898	
4	6		2.1165	
8	6			3.8378
Sig.		.070	.099	1.000

Means for groups in homogeneous subsets are displayed.

Cd

Descriptives

HM

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.0100	.00651	.00266	.0032	.0168	.01	.02
2	6	.0130	.00420	.00171	.0086	.0174	.01	.02
3	6	.0203	.00916	.00374	.0107	.0299	.02	.04
4	6	.0190	.00089	.00037	.0181	.0199	.02	.02
5	6	.0200	.00063	.00026	.0193	.0207	.02	.02
6	6	.0217	.00408	.00167	.0174	.0260	.02	.03
7	6	.0250	.00548	.00224	.0193	.0307	.02	.03
8	6	.0233	.00516	.00211	.0179	.0288	.02	.03
Total	48	.0190	.00681	.00098	.0171	.0210	.01	.04

ANOVA

HM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	7	.000	5.703	.000
Within Groups	.001	40	.000		
Total	.002	47			

HM

Duncan

SPP	N	Subset for alpha = 0.05		
		1	2	3
1	6	.0100		
2	6	.0130	.0130	
4	6		.0190	.0190
5	6			.0200
3	6			.0203
6	6			.0217
8	6			.0233
7	6			.0250
Sig.		.326	.054	.087

Means for groups in homogeneous subsets are displayed.

Cr

Descriptives

Cr

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.3030	.05215	.02129	.2483	.3577	.21	.36
2	6	.3270	.10600	.04327	.2158	.4382	.16	.45
3	6	.3155	.04432	.01810	.2690	.3620	.23	.35
4	6	.3293	.02448	.01000	.3036	.3550	.28	.35
5	6	.3230	.06595	.02692	.2538	.3922	.27	.45
6	6	.2302	.11776	.04808	.1066	.3537	.10	.43
7	6	.2107	.07271	.02968	.1344	.2870	.11	.31
8	6	.2078	.08643	.03528	.1171	.2985	.13	.35
Total	48	.2808	.08778	.01267	.2553	.3063	.10	.45

ANOVA

Cr

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.125	7	.018	2.999	.013
Within Groups	.237	40	.006		
Total	.362	47			

Cr

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
8	6	.2078	
7	6	.2107	
6	6	.2302	.2302
1	6	.3030	.3030
3	6		.3155
5	6		.3230
2	6		.3270
4	6		.3293
Sig.		.056	.055

Means for groups in homogeneous subsets are displayed.

Descriptives

Zn

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.4398	.26221	.10705	.1647	.7150	.20	.76
2	6	.3138	.15948	.06511	.1465	.4812	.06	.47
3	6	.1902	.05935	.02423	.1279	.2525	.11	.28
4	6	.0993	.03075	.01255	.0671	.1316	.07	.16
5	6	.1843	.14272	.05826	.0346	.3341	.03	.38
6	6	.1803	.17838	.07282	-.0069	.3675	.04	.45
7	6	.0685	.01854	.00757	.0490	.0880	.04	.09
8	6	.0903	.02871	.01172	.0602	.1205	.06	.12
Total	48	.1958	.17401	.02512	.1453	.2464	.03	.76

ANOVA

Zn

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.663	7	.095	4.985	.000
Within Groups	.760	40	.019		
Total	1.423	47			

Zn

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
7	6	.0685		
8	6	.0903		
4	6	.0993		
6	6	.1803	.1803	
5	6	.1843	.1843	
3	6	.1902	.1902	
2	6		.3138	.3138
1	6			.4398
Sig.		.189	.133	.121

Means for groups in homogeneous subsets are displayed.

Descriptives

SO2

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	1.2198E2	145.87417	59.55288	-31.1056	275.0656	30.19	415.00
2	6	90.5317	34.41759	14.05092	54.4126	126.6507	45.28	135.84
3	6	83.0150	24.80149	10.12516	56.9874	109.0426	45.28	120.75
4	6	62.8917	37.48017	15.30122	23.5586	102.2247	30.19	135.84
5	6	1.5849E2	73.17315	29.87281	81.6995	235.2805	60.38	241.52
6	6	2.1635E2	24.65085	10.06367	190.4789	242.2178	181.13	241.52
7	6	2.2389E2	25.99878	10.61396	196.6076	251.1757	196.22	271.69
8	6	2.0628E2	12.32420	5.03133	193.3482	219.2151	196.22	226.41
Total	48	1.4543E2	84.09139	12.13755	121.0112	169.8463	30.19	415.00

ANOVA

SO2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	175986.422	7	25140.917	6.431	.000
Within Groups	156367.592	40	3909.190		
Total	332354.014	47			

SO2

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
4	6	62.8917		
3	6	83.0150	83.0150	
2	6	90.5317	90.5317	
1	6	1.2198E2	1.2198E2	
5	6		1.5849E2	1.5849E2
8	6			2.0628E2
6	6			2.1635E2
7	6			2.2389E2
Sig.		.143	.062	.105

Means for groups in homogeneous subsets are displayed.

Descriptives

NO3

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	6	.0935	.03049	.01245	.0615	.1255	.05	.14
2	6	.1108	.04092	.01670	.0679	.1538	.07	.18
3	6	.1287	.06529	.02666	.0601	.1972	.04	.23
4	6	.0513	.01164	.00475	.0391	.0635	.04	.07
5	6	.1257	.09236	.03770	.0287	.2226	.04	.30
6	6	.0937	.03599	.01469	.0559	.1314	.05	.14
7	6	.0790	.01831	.00747	.0598	.0982	.05	.10
8	6	.1402	.08728	.03563	.0486	.2318	.07	.31
Total	48	.1029	.05837	.00842	.0859	.1198	.04	.31

ANOVA

SO2

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.036	7	.005	1.671	.144
Within Groups	.124	40	.003		
Total	.160	47			

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
4	6	.0513	
7	6	.0790	.0790
1	6	.0935	.0935
6	6	.0937	.0937
2	6	.1108	.1108
5	6		.1257
3	6		.1287
8	6		.1402
Sig.		.105	.106

Means for groups in homogeneous subsets are displayed.