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THE USE OF LICHENS AS BIO-INDICATORS AND A COST-EFFECTIVE WAY FOR HEAVY METALS POLLUTION MONITORING IN AMBIENT AIR IN THE WESTERN AREA AND SOUTHERN PROVINCE OF SIERRA LEONE

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Abstract

With increasing economic crises around the world, more so for countries like Sierra Leone where the application of state-of-the-art laboratory facilities are lacking due to the virtual lack of resources, the use of low-cost efficient technology is a suitable alternative. In light of this, the use of Lichens as a bio-indicator of air quality for heavy metals across the Western Area (Urban and Rural Areas) and Southern Province (Bo and Bonthe Districts) in Sierra Leone was investigated. Heavy metals concentrations in Lichen species were determined across several study locations using The Energy Dispersive X-Ray Fluorescence (EDXRF) and Atomic Absorption Spectroscopy (AAS). Lichen samples were collected from trees back having 30 - 70 cm in diameter and height of 2 - 5 meters. The dried unwashed samples were digested to analyze the concentrations of heavy metals at the Institut National des Sciences et Techniques Nucleaires (INSTN) Campus University Ankatso Antananarivo Madagascar. The Graphis species in the western urban area (Kingtom power station) exhibited accumulation of heavy metals: Cr (2.3 mg/kg), Co (2.7 mg/kg), Cu (22.4), Fe (1383.3 mg/kg), Pb (4.1 mg/kg), Ni (17.4 mg/kg) and Zn (46.6 mg/kg) and the Ocellularia species in the western rural area (Black Johnson) exhibited accumulation of heavy metals: Cr (1.0 mg/kg), Co (0.5 mg/kg), Cu (7.9 mg/kg), Fe (321.9 mg/kg), Pb (0.5 mg/kg), Ni (6.2 mg/kg) and Zn (5.0 mg/kg) the most prevalent in polluted and clean air environs respectively. The southern province (Bo and Bonthe districts) exhibited a high concentration of pollutants. The Arthonia species in the Bo district (Kebbie Town) exhibited accumulation of heavy metals: Cr (1.5 mg/kg), Co (2.3 mg/kg), Cu (15.7), Fe (1333.5 mg/kg), Pb (1.8 mg/kg), Ni (11.45mg/kg) and Zn (16.9 mg/kg) whilst Diorygma species in the Bonthe district (Petema Town) exhibited accumulation of heavy metals: Cr (1.0 mg/kg), Co (0.8 mg/kg), Cu (12.7 mg/kg), Fe (1593.8 mg/kg), Pb (1.1 mg/kg), Ni (6.2 mg/kg) and Zn (9.3 mg/kg). The high accumulation of pollutants was attributed to the high carbon monoxide concentration from industrial operations, frequent vehicular movement, and pollution associated directly with human activities through the burning of fossil fuel and other materials. The level of heavy metal pollutants in the Lichen species mirrored those obtained from active sampling using air pollution equipment in the Western Area. The results support the idea that a lichen biomonitoring approach can be used as an effective low-cost tool for monitoring heavy metal pollution in air and provided essential baseline information for future studies on the effect of air pollution on lichen communities in Sierra Leone.

Keywords: Air quality, Bio-indicator, heavy metals, Lichens and low-cost

Introduction

Air pollution is a significant global problem with upraised concentrations of pollutants reported from many parts of the world. Air pollution is caused by the release of particulate matter from mainly anthropogenic emissions into the atmosphere. A study

investigated the particulate matter concentration (PM_{2.5} and PM₁₀) for both indoor and outdoor air in specified locations in the city of Freetown, Sierra Leone. Results indicated that the west end of the city have a better air quality compared to the east and central part of the city (Kargbo and Frazer-Williams, 2016; Taylor and Nakai, 2012). Furthermore, there was a positive correlation between indoor and outdoor air quality. In general, the air quality in some parts of the city of Freetown was considered to be relatively poor and in assessment of mortal exposure, large percentage of residential areas in the city are open to high concentration of PM which is considered a public health risk. (Kargbo and Frazer-Williams, 2016). This and other interventions by the Environment Protection Agency Sierra Leone (EPASL) has raised the awareness of air pollution in Freetown and ways to combat it. It is a step towards joining many countries who setup nationwide monitoring programmes for ambient air monitoring which is systematic, long-term assessment of pollutant levels by measuring the quantity and types of certain pollutants such as carbon monoxide, nitrogen dioxide, ozone, sulphur dioxide, PM_{2.5} and PM₁₀. (Josipovic et al., 2010; Plaia & Ruggieri, 2011).

Air pollution occurs due to natural phenomenon as well as human activities such as emission of harmful gases and dust particles from vehicles, combustion of fossil fuels like coals, petroleum releasing oxides of nitrogen and sulphur and smoke release from industries containing harmful gases like sulphur dioxide, oxides of nitrogen (Shukla and Upreti, 2011). Amongst the various emission sources, industrialization is one of the biggest reasons behind the increase of air pollution and it has been reported for its potential dangers in plants, animals and human beings for decades. (Sacks, Stanek, Luben, Johns, Buckley, Brown, et al 2011). Air pollution comprises of pollutants such as carbon monoxide, lead, nitrogen oxides, ground-level ozone, particle pollution (often referred to as particulate matter), and sulfur oxides. The composition of pollutant air is not constant and changes from place to place. In polluted areas, emissions contains very high pollutant concentrations.

Particulate matter is the sum of all solid and liquid particles suspended in air many of which are hazardous. This complex mixture includes both organic and inorganic particles, such as dust, pollen, soot, smoke, and liquid droplets. These particles vary greatly in size, composition, and origin which can penetrate deeply into the lung, irritate and corrode the alveolar wall, and consequently impair lung function, children and older adults are the most likely to be affected by particle pollution exposure. (Environ Health Perspect 2011 Apr; 119(4):446-454.). The rate and level of air pollution in the atmosphere is getting increasingly threatening to life on earth. (Environmental Monitoring and Assessment 131 (1), 365-369, 2007Shukla V and Upreti DK. 2007).

The Environmental Protection Agency (EPA) maintains a repository of air quality data in accordance with the Air Quality system (AQS), there are five (5) main methods of sampling air quality and these are:

- Passive monitoring
- Active (Semi-Automatic) sampling
- Automatic point monitoring
- Photochemical and optical sensor system
- Remote optical/long-path monitoring

Biological monitoring (or biomonitoring) describes the use of bioindicators to evaluate various environmental conditions. Lichens have been found to be bioindicators for the general atmospheric conditions since they absorb water and nutrients from the air (including pollution) over their entire surface. Lichens are well known as sensitive indicators of air pollution, this is related to their unique biology.

Lichen is a Greek word which means the superficial growth on the bark of olive trees. In 1867, the dual nature of lichens was discovered by a Swiss botanist Simon Schwendener and it was a really unexpected disclosure in biology. The scientific society has been debating on the nature of lichens until today. The name lichen refers to its mycobiont, since 1983 (Voss et al., 1983). Lichens are symbiotic organisms that are formed by mutual relationship of algae and fungi. Mostly Ascomycota (98%) is fungal partner in lichens (Gilbert, 2000; Honegger, 1991) and the other belong to the Basidiomycota and anamorphic fungi. Lichens have been recognized as valid tools for evaluation of air quality based on physiological and morphological characters (Nimis & Mertellose, 2002). Lichens can grow in different climatic conditions and on different substrates. The lichens which grow on other plants are called epiphytic, tree trunks and barks are called corticolous, twigs are called ramicolous, mosses are called muscicolous, dead woods are called legnicolous, rocks and boulders are called saxicolous (epilithic) and on soils are called terricolous (Batic & Mayrhofer, 1996); (Gustafsson et al., 2004). Lichen grow slowly, long-lasting, the important parameters required for the growth and abundance are adequate amount of moisture, light, altitude and unpolluted air (Costa et al., 2002). The biological monitoring methods are fast and cheap (Markert et al., 2003); (Begu, 2011). According to the literature (Puckett, 1988), lichens are considered to be the best body to be used as bio-indicators of air pollutants. The use of lichens as an indicators of air pollution has been studied in Europe and Northern America (Loppi & Frati 2006, Thormann 2006, Pinho et al., 2004), however, little is known about air pollution and its effects on lichen in Africa.

In Sierra Leone, biomonitoring studies with lichens was initiated for the first time as no record on studies on both organic and inorganic metals and other pollutants using Lichen as biomonitor are available for the country. However, in the present investigation, studies have been made to assess the status of metal pollution around mineral processing plant and traffic areas in the southern province districts, with the help of a foliose lichen; which grows abundantly on variety of trees.

Literature reveals that among the different lichen species, members of lichen family Physciaceae (*Pyxine* sp., *Dirinaria* sp., *Phaeophyscia* sp.) were used frequently for biomonitoring (Bajpai et al. 2010a, 2011, Satya et al. 2009, Shukla & Upreti 2007, Bajpai et al. 2004); therefore, in the present study, *Dirinariaaapplanata* species are used.

Materials and Methods

Sample Collection

The micro or macro lichens are visible to the naked eyes in the field. However, a hand lens was used to examine the structure of the thallus and confirm while collecting the lichens. A sharp hard knife, flat edged chisel (1 to 2 inch) and a hammer (1 to 2kg weight) were the tools use to scrape and collect the lichen samples from the back of the trees. The lichens were collected and placed inside a brown envelop with it appropriate labeling.

Table 1: Locations and general air quality where Lichen species were collected in Sierra Leone

Site	Locations	Air quality	Remarks	GPS	
				N	W
Site 1	Imperi Chiefdom, Bonthe District	Poor	Mineral processing plant area with trucks hauling ore materials.	7°52'11	12°28'15
Site 2	Bo city, Bo District.	Moderate	Anthropogenic pollution and less traffic visibility.	8°00'00	11°39'90
Site 3	Aberdeen and Spur Loop, Western Area Urban	Good air	Neatly maintained residential area.	8°28'37	13°17'22
Site 4	Beach view area, Western Area Rural	Very clean air	Foreshore area	8°15'25	13°9'32

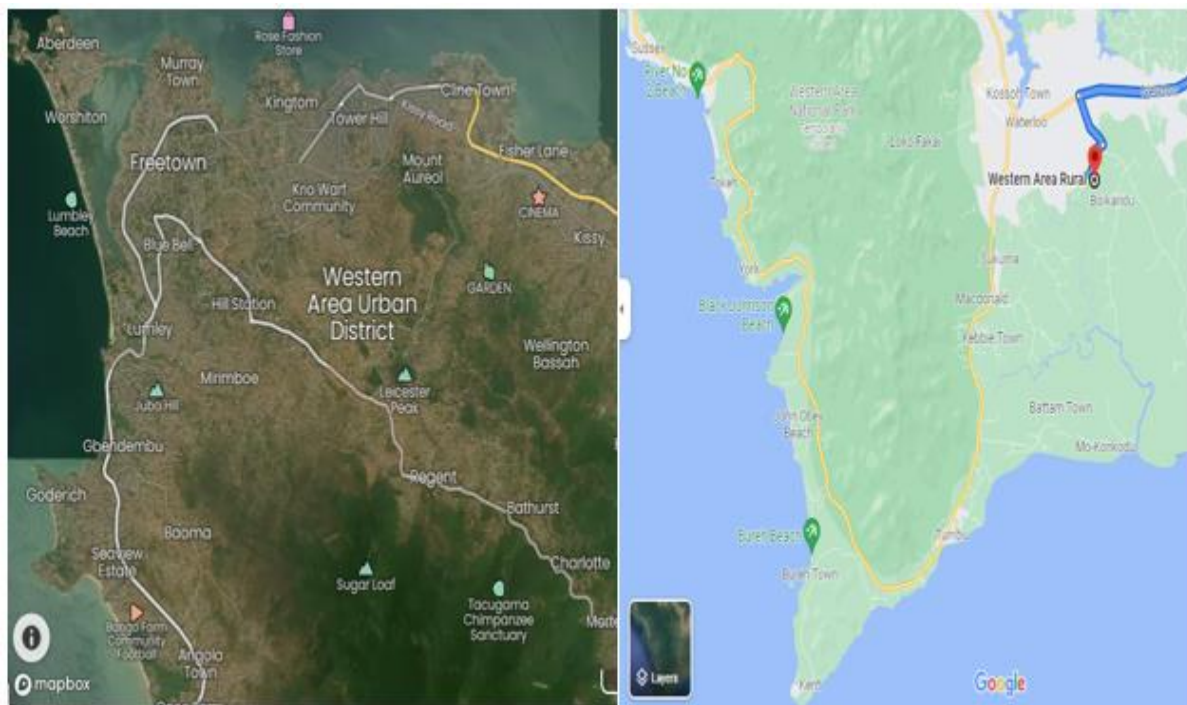


Fig. 1. Lichens sampling points in Western Urban & Rural localities in and around Freetown
(The map was exported from Google Earth).

Description of sample preparation

Laboratory PPEs (nitrile gloves, coat, and google) were worn and samples were handled with care. Each sample was examined using a dissection microscope for confirmation

Drying and Grinding

Lichen samples were dried in the oven at 60°C for 6 hours. The dried samples were then grounded with a Spex mixer/miller for about 10-15 min. Each sample weight was recorded before and after drying to determine the relative moisture content.

Wet digestion methods

Approximately 0.5g of the dried lichen sample was transferred into a 50 mL volumetric flask. 6 mL of nitric acid (65% HNO₃) was added and heated on a hot plate at 100°C ± 5°C for 10 min and then at 150°C ± 5°C for 30 min. After cooling down, 2 mL of 70% perchloric acid (HClO₄) was added and the solution was heated again until it becomes colourless and clear. After cooling, the digested sample in the 50 mL flask was filled with deionized water to the calibration mark.

Determination of metal contents in samples

Energy Dispersive X-Ray Fluorescence (EDXRF): Upon arrival in the laboratory, the lichen samples were washed with milli-q water to remove any dust within the sample. After 24 hours of air-drying, the samples were put in an electrical oven at temperature of 60°C for 2 hours to dry the samples, then grinded with mortar and pestle. The powdered sample was sieved with 150µm particle size sieve shaker. 3 g from the sieved samples were weighed with analytical balance (Mettler Toledo AG 285) and pressed with press pellet machine to get pellets samples. These pellets samples were used for the elemental analysis by the EDXRF.

Atomic Absorption Spectroscopy (AAS): The sample preparation for the AAS is the same as the EDXRF method except after sieving the samples with 150µm particle size sieve shaker, 0.3 g of the samples were weighed with analytical balance (Mettler Toledo AG 285) and transferred into a digestion flask. 4.2 g of nitric acid and 1 g of hydrogen peroxide were added into each flask containing the sample; each flask lagged with bomb calorimeter and placed in an oven at temperature of 165°C for 6 hours for the digestion process. Digested samples were diluted to 20ml with de-ionized water. The diluted samples were analyzed for heavy metal concentration using AAS.

Technical Information on the EDXRF and AAS used in the Analysis

The EDXRF used for the elemental analysis in the present study was the SPECTRO XEPOS III. This spectrometer uses a palladium or cobalt target end window tube at a maximum power 50 W and voltage 50 kV with vacuum cooling to excite the pellet samples. The target of this spectrometer changer with up to eight polarization and secondary targets such as Mo, Co, Pd, Zn, Zr, CsI, highly oriented pyrolytic graphite and aluminum oxide (Al₂O₃). This principle offers many different excitation conditions ensuring optimum determination of all elements detectable in the samples.

The Atomic Absorption Spectrometry (AAS) used in the present study was the AA240FS and AA240Z. These two spectrometers were used to analyze the concentration of the elements in the digested samples. The atomic absorption spectrometry analytical method is one of the most used techniques for the quantitative determination of elements in environmental materials at trace and ultratrace levels. AAS is an optical atomic spectrometric technique based on the measurement of the specific absorption originating from free non-ionized atoms in the gas phase. To transfer the analyte to free atoms, different types of atomizer are in use, the flame and the graphite furnace was used during the analysis. Typical detection limits of flame atomic absorption spectrometry (FAAS) are of the

order of 1–100 $\mu\text{g L}^{-1}$, making it a perfect tool for the determination of minor and trace elements, at least for contaminated samples. Graphite furnace atomic absorption spectrometry (GFAAS), offering detection limits which are about a factor of 20–200 lower than for FAAS, is the standard method for many trace elements, especially for background values. AAS in its conventional configuration is a single-element technique, which must be used in a sequential mode when more than one element has to be determined.

The results presented in this work were obtained from samples collected between April - November 2019. The trees for the lichen sampling were chosen from the areas with different pollution levels, collected from the following areas: near mineral processing plant which produce gaseous pollutants, anthropogenic pollution with less traffic visibility, neatly maintained residential areas, botanical garden and beach view areas. Thus, a comparative analysis between heavy metal air pollution can be made from the various sites. Samples records were taken for sampling dates, locations and GPS coordinates.

Results and discussion

(i) Lichens Survey

The survey examined forty-nine mature-grown lichen trees with diameters greater than 76 cm at chest height (Table 2). The trees that met the criteria for selection were found in all sampling sites. Twenty species were identified in the Western Urban Area, nine species were identified in the Western Rural Area, eight species were identified in the Bo District (Bo Town) and twelve species were identified in the Bonthe District (Imperi Chiefdom).

Lichen *Graphis* species *thalis* were identified to be abundant. The most common species identified in all sampling sites during the survey are shown in Table 2. Figure 2 show the common and all lichens surveys during the studies.

Table 2: Total number of species surveyed

Lichen species	Total Survey
<i>Arthonia cinnabarina</i> (DC.) Wallr. sp.	3
<i>Arthonia</i> sp.	3
<i>Astrothelium cinnamomeum</i> (Eschw.) Cinnamomum. sp.	1
<i>Bacidina</i> indet sp.	1
<i>Diorygma</i> sp.	5
<i>Fellaneropsis</i> sp.	1
<i>Graphis</i> sp.	7
Indet sp.	1
<i>Leptogium austroamericanum</i> (Malme) sp.	2
<i>Leptogium</i> indet.	1
<i>Leucodecton</i> sp.	3
<i>Malmidea ceylanica</i> (Zahlbr.) Kalb & Lumbsch	2
<i>Myriotrema</i> sp.	1
<i>Ocellularia</i> sp.	3
<i>Opegrapha</i> indet sp.	1
<i>Phaeographis</i> cf. <i>intricans</i> sp.	1
<i>Phaeographis</i> sp.	1

Phyllopsora sp.	1
Physcia atrostriata Moberg sp.	1
Platythecium sp.	1
Porina internigrans (Nyl.) Müll. Arg. sp.	3
Pseudopyrenula endoxanthoides Vain. Sp.	1
Pyrenula mastigophora Aptroot sp.	1
Sarcographa labyrinthica (Ach.) Müll. Arg. Sp.	1
Sorediate Graphidaceae sp.	2
Sterile crust	1

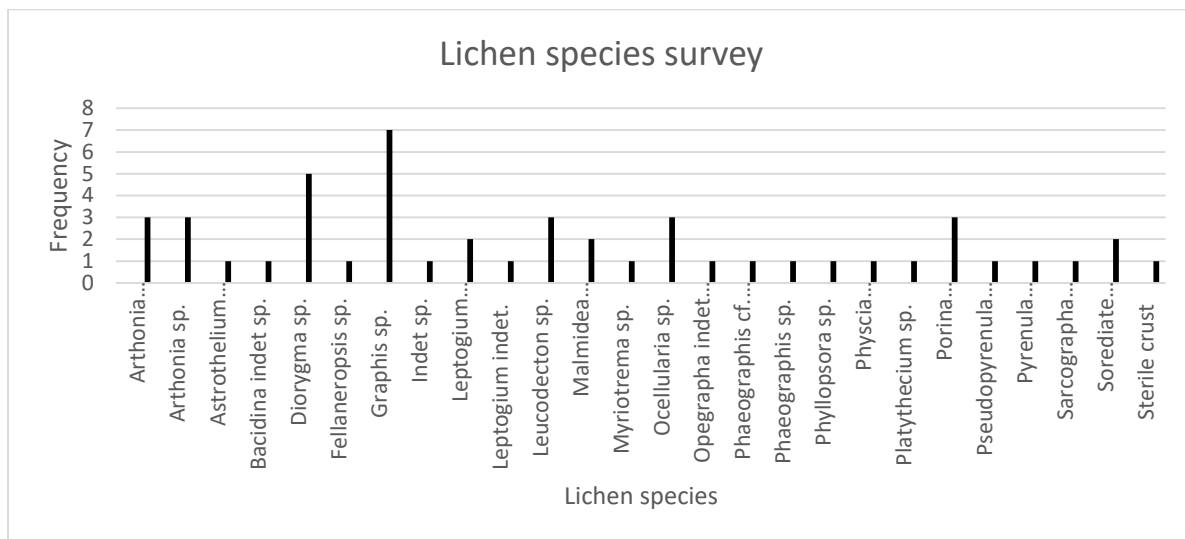


Figure 2: Lichen species frequency

(ii) Lichen Diversity

Lichens are symbiotic associations between a fungus and one or more photosynthetic partners. These organisms lack protective tissues, so they readily absorb water, nutritive substances, and gases directly from the atmosphere. Due to these physiological peculiarities, lichens are sensitive to a suite of anthropogenic disturbances, such as atmospheric pollution, climate change, or forest management. (Bajpai R, Upreti DK, Dwivedi SK. 2010 – Passive monitoring of atmospheric heavy metals in a historical city of central India by *Lepraria lobifera* Nyl. Environmental Monitoring and Assessment 166, 477–484.) The lichen species depend on pollution pattern, some sensitive lichen species develop structural changes in response to air pollution including reduced photosynthesis and bleaching. Pollution can also cause the death of the lichen algae, discoloration and reduced growth of the lichen fungus, or kill a lichen completely.

Foliose lichen of *Dirinaria appplanata* were collected and analyzed for heavy metal identification. It was observed that there are variation in lichen diversity in different localities. The abundance of the lichens were observed at Site – 4 Western Rural Area (Foreshore areas) and Site – 3 Western Urban Area (neatly maintained residential areas). This is attributed to the presence of a non-polluted atmosphere with moist and shady condition suitable for lichens. Less number of lichen species were observed at Site – 2 Bo city (Bo District) and Site – 1 Imperi chiefdom (Bonthe District). This is attributed to the polluted atmosphere prevalent in these areas, due to industrial processed, residential anthropogenic, vehicular movement and agricultural systems.

(iii) Heavy Metal Concentrations in Lichens

The level of accumulation of heavy metals in Lichens from the study sites depends on the pollution status of the study areas. Site - 1 (Imperi chiefdom, Bonthe District) is a mineral processing plant area with trucks hauling ore materials, Site - 2 (Bo city, Bo District) is an anthropogenic polluted area, less traffic visibility and exposed for commercial exploitation, Site - 3 is a neatly maintained residential area with no traffic visibility and Site - 4 (Western Rural) is a beach view area associated with sea breeze and quietness. The lichens collected from Fourah Bay College, University of Sierra Leone botanical garden were initially estimated to be free from the accumulation of heavy metals and observed that it was below the threshold level (Table 3).

Table 3 Heavy metal concentration in the thalli of lichen *Dirinariaaapplanata* collected from Fourah Bay College, University of Sierra Leone Botanical Garden. All values are mean \pm S.D. (n=3)

Metal	Accumulation (mg/kg)
Cadmium	<0.08
Chromium	<1.02
Cobalt	<0.05
Copper	7.94 \pm 0.90
Iron	321.97 \pm 19.95
Lead	0.51 \pm 0.01
Nickel	6.19 \pm 0.26
Zinc	4.96 \pm 0.09

Table 4 Metal accumulation at different sites. Values are mean \pm S.D. (n=12)

S. No.	Metals	Values (mg/kg)			
		Site 1 Bonthe District	Site 2 Bo District	Site 3 Western Urban	Site 4 Western Rural
1	Cd	<0.08	0.18 \pm 0.01	<0.08	<0.08
2	Cr	<1.02	2.22 \pm 0.05	1.17 \pm 0.03	<1.02
3	Co	<0.05	1.68 \pm 0.30	2.46 \pm 0.03	1.66 \pm 0.28
4	Cu	14.78 \pm 1.43	13.54 \pm 0.97	12.83 \pm 1.10	8.50 \pm 0.92
5	Fe	2844.80 \pm 101.74	2162.65 \pm 63.03	271.77 \pm 25.32	133.49 \pm 21.85
6	Ni	<4.58	10.07 \pm 0.32	13.76 \pm 0.42	11.66 \pm 0.32
7	Pb	4.27 \pm 0.41	2.82 \pm 0.29	0.68 \pm 0.02	<0.23
8	Zn	38.00 \pm 3.49	18.22 \pm 2.49	4.63 \pm 0.11	6.67 \pm 0.13

It was observed that accumulations of heavy metals were high in Site - 1 (Imperi chiefdom, Bonthe District) (Table 4) and exhibited higher levels of the metals (Cu, Fe, Pb, and Zn) compared to the other sites of study with exception of Cd, Cr, Co and Ni which are low in concentration. The probable reason for higher concentration of different metals in the area is due to the presence of the mineral processing plant and haulage trucks which emitted pollutant gases. Similar findings within the central India, accounts of lichen biomonitoring studies with lichens initiated along industrial and traffic rich areas in coastal Cuddalore

district, with the help of a foliose lichen which grows abundantly in mangroves. Active monitoring employs the transplantation of lichens from healthy site to polluted site, to determine the level of pollution or accumulation reported by Dubey et al. (1999), Upreti & Bajpai (2001), Bajpai et al. (2010a).

Cadmium is one of the most hazardous elements and it is released into the environment by fossil fuel combustion, production of phosphate fertilizers and municipal solid waste incineration (Morrow 2010). Further, Rani et al. (2011) reported *Dirinariaapplanata* accumulated cadmium (Cd) ranging from 532 mg/kg to 875 mg/kg. However, in the present investigation all sites were free from toxic cadmium, cadmium level was recorded higher at Site - 2 (Bo city, Bo District) (0.18 ± 0.01 mg/kg). The absence of phosphate fertilizers and municipal waste incinerator in these areas and also the country possibly could explain the low value of Cd in lichens reported in this study.

The presence of chromium (Cr) in lichen thallus exhibits its airborne origin, and it is emitted in the atmosphere by different sources like coal and oil combustion, steel manufacturing and cement production (Shtiza et al. 2008).

In the present investigation, concentration of chromium (Cr) ranged from <1.02 to 2.22 ± 0.05 mg/kg at Site - 2 (Bo city, Bo District). The higher chromium (2.22 ± 0.05 mg/kg) exhibited may be due to the pollution associated directly by human activities through burning of wood materials. Similarly, literature also reveals that *Dirinariaapplanata* can accumulate 61.80-1920 mg/kg chromium (Mishra et al. 2003, Bajpai et al. 2004).

According to Baptista et al. (2008), iron (Fe) content in lichens is evidently affected by iron originating from fuel and soil dust. Lichens have special affinity with iron, and most of the lichens species accumulate the metal in higher concentration than all the other metals. Majority of the previous findings carried out with lichens, regarding metal accumulation, iron (Fe) has exhibited higher concentration. (Nayaka et al. 2003, Shukla et al. 2007). *Dirinariaapplanata* accumulates higher amount of iron ranging from 1573.0 - 19374.0 mg/kg (Saxena et al. 2007). Similarly, in the present study, iron was accumulated in higher concentrations at all the sites especially at Site - 1 (Imperi chiefdom, Bonthe District), it was 2844.80 ± 101.74 mg/kg due to ore processing plant and other activities.

As per the literature, lead (Pb) content with traffic volume is directly proportional to Pb accumulation in lichen thallus (Takala & Okkonen 1981). *Dirinariaapplanata* accumulates higher amount of Pb ranging from 8600 ± 395 to 12433 ± 185 mg/kg (Rani et al. 2011). However, the tyres activity and application of brake pads release Zinc (Zn) in the environment (Bajpai et al. 2011). Further, it was also reported that *Dirinariaapplanata* accumulates higher concentration of zinc than the other lichens (Bajpai et al. 2011).

Similarly, in the present findings, maximum accumulation of Pb (4.27 ± 0.41 mg/kg) and Zn (38.00 ± 3.49 mg/kg) were found at Site - 1, as the site showed heavy vehicular activity with emission of pollutant gases, followed by Site - 2 (Bo city, Bo District) Pb (2.82 ± 0.29 mg/kg) and Zn (18.22 ± 2.49 mg/kg) which also have vehicular activities. Zinc chloride is a chemical which is used in the manufacturing of tyres of motor vehicles, and this may be the reason for the accumulation of zinc in the lichens as Site - 1 (Imperi chiefdom, Bonthe District) and Site - 2 (Bo city, Bo District) as they are traffic prone areas than the other two sites.

Furthermore, the concentration of Cu, Fe, Pb, and Zn was recorded maximum at Site – 1(Imperi chiefdom, Bonthe District) in comparison to the other three sites. Higher accumulation of most of the metals may be because of the presence of the mineral processing plant and other activities which emitted various types of contaminants into the atmosphere. Moreover, the metals in lichens ranged as follows: Fe>Zn>Cu>Ni>Pb>Cr>Co>Cd.

Conclusion

Since the lichens are excellent bio-accumulators of heavy metals, it is used in the biomonitoring studies as well as to detect the metallic and organic pollutants from the environment. Active monitoring studies have been carried out with foliose lichens *Dirinariaaapplanata* species *a* clearly proved that *Dirinariaaapplanata* is a good accumulator for biomonitoring of air quality. Hence from the present study, it was concluded that the accumulation of heavy metals at Site – 1(Imperi chiefdom, Bonthe District) lichen thallus indicates that Imperi chiefdom, Bonthe District has higher contaminated with the heavy metals. The higher accumulation of Cu, Fe, Pb and Zn indicates the hazardous status of the study area.

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