Quantification of Heavy Metal Deposition in Lichen Species of Kathmandu Valley

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ABSTRACT

Heavy metal pollution in urban areas is the major concern of every rapidly growing nation. The main aim of this study was to determine the heavy metal concentration around Kathmandu valley using locally growing lichens species. The study was conducted by random sample collection method throughout Kathmandu, at the interval of 2km considering Tri-Chandra College as reference point in four geographical directions based on radial distribution pattern. In this study, we quantified Lead (Pb), Cadmium (Cd), and Chromium (Cr) in *Pyxine cocoes, Arthopyrenia sp, Caloplaca sp,* and Hypotrachya revolute collected from Kathmandu valley. The samples were subjected to Atomic Absorption Spectroscopy (AAS) for heavy metal determination. High concentrations of heavy metals were detected in areas that experience a high influx of vehicles like the Swayambhu area, Pepsicola area, and Lagankhel area. The areas further away from heavy traffic roads showed a significantly low amount of heavy metal concentration. The study provides an insight into the current state of heavy metals in Kathmandu valley, which in high concentration can be detrimental to the health of living organisms.

Keywords: AAS, Bio-indicator, Cadmium, Chromium, Lead, Lichens.

1. INTRODUCTION

Lichens have a well-established reputation as a good biomonitor. These symbiotic organisms consisting of a fungal partner 'mycobiont' and a photosynthetic partner 'photobiont' can survive in an extensive range of micro and macrohabitat (Nash 2008). Lichens are very sensitive to change in air temperature, humidity, wind, and air pollutants because they don't have cuticles as vascular plants, thus absorbing water and nutrients passively from their surrounding environment. Lichens are highly sensitive, which react to slightly elevated levels of surrounding which meet several requirements of the ideal biomonitor; large geographical ranges allowing comparison of metal concentration from the diverse region; a morphology which does not vary with seasons, thus enable accumulation to occur throughout the year. Both essential and nonessential elements are bioaccumulated in lichens through various mechanisms, including surface complexation, biomineralization, and physical trapping of dust and soil particulates in the intercellular spaces of the medulla (Nash 2008). Absorption takes place through the whole surface of the thallus so that elements present in the atmosphere and substrate can penetrate the lichens thalli (Shaw 1989).

Increasing pollution is a significant concern in our modern society. Industrialization and technological advancement have put an increasing burden on the environment by releasing large quantities of hazardous waste, heavy metals (cadmium, chromium, and lead), and metalloids (elements with intermediate properties between those of typical metals) non-metals (Ayangbenro & Babalola 2017). Heavy metals cannot be degraded naturally by chemical and biological processes. Hence, they persist in toxic amounts in nature, threatening the health of living organisms (Ayangbenro & Babalola 2017). The naturally occurring heavy metals are not readily available for plants, whereas the bioavailability of anthropogenic sources of heavy metal is very high because of their solubility and reactive form. These anthropogenic sources include alloy production, atmospheric deposition, battery production, biosolids, coating, explosive manufacturing, improper stacking of industrial solid waste, leather tanning, mining, pesticides, phosphate fertilizer, photographic materials, printing pigments, sewage irrigation, smelting, steel, and electroplating industries, textiles, and dyes and wood preservation (Ayangbenro & Babalola 2017).

Heavy metals' main threats to human health are exposure to lead, cadmium, mercury, and arsenic. These metals have been extensively studied, and their effects on human health are regularly reviewed by international bodies such as the WHO (Järup 2003). Heavy metals can bind to vital cellular components, such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning. Broadly, long-term exposure to heavy metals can have carcinogenic central and peripheral nervous system and circulatory effects (Ugwu 2018). Cigarette smoking is a significant source of cadmium exposure. In non-smokers, food is the most important source of cadmium exposure. Recent data indicate that adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily in the form of kidney damage but possibly also bone effects and fractures (Ebert-McNeill *et al*. 2012). Long-term exposure to arsenic in drinking water is mainly related to increased risks of skin cancers. As well as the skin lesions such as hyperkeratosis and pigmentation changes (WHO 2023).

Similarly, lead poisoning runs havoc in our body. Exposure to high levels of lead can disrupt renal, reproductive, hematopoietic and central nervous system(Assi *et al*. 2016). Chromium falls under category 1 carcinogen which can damage our organs like skin, liver, kidney and respiratory organs. Dermatitis, lung cancer, etc are some examples of the disease caused due to exposure to chromium.(Zhong, Ren, & Zhao 2016)

Nepal is rapidly urbanizing, and the number of people living in urban areas, especially Kathmandu, has significantly increased. As most industrial plants are in the valley, along with rapidly growing vehicle count, pollution in Kathmandu is increasing heavily(Saud & Paudel 2018). Biological monitoring can be an effective early warning system to detect environmental changes (Markert *et al*. 2003). In samples collected from

Sivapuri Watershed and Wildlife Conservation Area, which lies in the northern part of the valley, the heavy metal concentration of 7.885 µg/g Pb in Parmelia simplicior and 0.378 µg/g Cd in Lobaria retigera were recorded (Devkota *et al*. 1997). Similarly, the study conducted to observe toxic metal accumulation in lichens through transplantation, and passive monitoring methods in Kathmandu showed a correlation between high levels of heavy metal accumulation in lichens with dense traffic areas (Chettri *et al*. 2015). However, the study was conducted in heavy traffic areas exclusively, not considering inner regions where heavy metals disperse through the air. The main aim of this study was to obtain the heavy metal accumulation throughout the Kathmandu valley at central points in all directions to include all the areas and account for heavy metal decomposition through diffusion.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted around the Kathmandu valley. Taking Tri-Chandra college as reference (central point), samples were collected approximately 2km apart in four geographical directions (East, West, North, and South) based on radial distribution pattern (Fig. 1). Kathmandu Valley lies in a warm temperate zone (elevation ranging from 1,200 to 2,300 meters), where the climate is relatively temperate, atypical for the region. The average summer temperature in Kathmandu Valley varies from 28 to 30 °C, and the average winter temperature is 10.1 °C. Rainfall is primarily monsoon-based (about 65% of the total concentrated during the monsoon months of June to September) with an average humidity of 75% (Asian Hiking Team 2023).

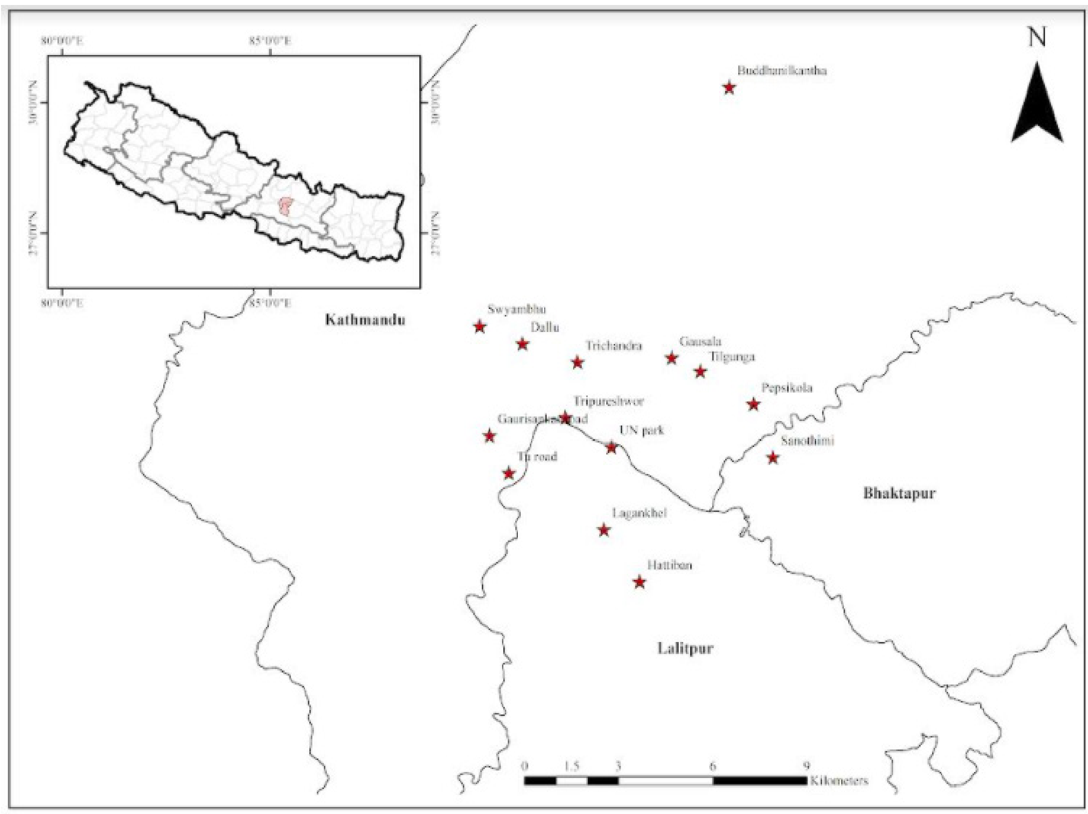

Fig.1: Study area and sites

Each point in the map (Fig. 1) is GPS coordinates from where samples were collected.

2.2 Sample Collection

Samples were collected in the cold winter during March 2017, when there was no significant rain or strong wind for at least a week beforehand. A total of 23 samples were collected. Mainly epiphytic lichens growing in trees close to the main road were selected. Vehicles were a significant source of air pollution; hence trees near the road, where lichens were exposed to air pollution all around the year were selected. The sampling points were recorded with GPS positioning logging their latitude and longitude. Lichen species observed on each tree sampled were recorded along with tree diameter, sample elevation in the tree from the ground, sample orientation. Lichen samples from bark were carefully taken using a knife and chisel then stored in a pre-labeled lichen pouch in the voucher.

2.3 Identification of Lichen Samples

A voucher sample of each lichen was prepared after bulk collection of that same sample from that particular study area. Records of GPS information, place or area of collection, habitat, name or type of host, and dates were recorded for each voucher specimen. All specimens were air-dried after being brought to the laboratory and pressed adequately before making their herbarium. A well-labeled herbarium was prepared for taxonomic identification of each sample as the method followed (Devkota *et al*.1997).

Taxonomic identification of each voucher specimen was made at the lichenological laboratory, Central Department of Botany, Kirtipur. Morphological characters, life forms, internal anatomy, and color spot tests were applied to identify each lichen sample as the method prescribed in (Awasthi 2007; Singh *et al*. 2019). The color test was done by using a small drop of an aqueous solution of Potassium hydroxide (KOH), Calcium hypochlorite ($CaOCl_2$), and Para-phenylenediamine on the thallus either separately or in associate as taxonomic key demands (Awasthi 2007). Each specimen was later confirmed after authentic identified samples were deposited in the lichen herbarium of the Central Department of Botany.

2.4 Sample Preparation

The samples were taken to the lab and air-dried for two days, covered in cardboard to prevent moisture and wind. Foreign materials (dust and debris) from the sample were removed using a scalpel and brush. The clean sample was now grinded to powder form. About 1gm of powdered sample was taken in a crucible and kept in the furnace at 500°C for 10-12 hrs. The ashes of the sample were cooled and placed in an acid-washed beaker. About 2.5 ml of concentrated nitric acid (HNO_3) was added to the ashes. The apparatus was placed on the hot plate and allowed to digest for 3 hrs. After digestion, distilled water was added to maintain 10cc volume. The digest was then filtered. The filtrate was subjected to Atomic Absorption Spectroscopy (AAS) for heavy metal determination.

2.5 Instrumentation

A novAA350 (Analytik Jena, Jena, Germany) flame technique atomic absorption spectrometer was used for the analysis of samples. The method was optimized based on the proper atomic lines for Pb (283.306 nm), Cd (228.802 nm), Cr (357.869 nm). The AAS machine was calibrated with the calibration solutions prepared by the certified reference materials (CRMS) of respective metals manufactured by Sigma Aldrich. An Ohaus electronic balance was used to weigh samples.

2.6 Statistical Analysis

To validate the significance of data, one way ANOVA (<0.05) test was also performed.

3. RESULTS

Among 23 samples collected from Kathmandu valley, four species of lichens were identified with the most frequent occurrence of Pyxine cocoes, followed by *Arthopyrenia sp* (Table 1). The concentration of heavy metals (Pb, Cr, and Cd) in the lichens is shown in (Table 1).

Table 1: Heavy metal concentration observed in samples

SN	Location	Lichen Species	Pb (µg/g) ± SD	Cr (µg/g) ± SD	Cd (µg/g) ± SD
1.	Budhanilkantha(a)	*Pyxine cocoes*	2.45±2.49	3.19±1.82	0.32±0.23
2.	Budhanilkantha(b)	*Pyxine cocoes*	3.06±2.49	0.95±1.82	0.47±0.23
3.	Tilganga(a)	*Arthopyrenia sp*	5.24±2.49	2.92±1.82	0.69±0.23
4.	Tilganga(b)	*Arthopyrenia sp*	6.55±2.49	3.26±1.82	0.37±0.23
5.	Kirtipur(a)	*Arthopyrenia sp*	4.86±2.49	0.49±1.82	0.28±0.23
6.	Kirtipur(b)	*Arthopyrenia sp*	8.14±2.49	1.32±1.82	0.58±0.23
7.	Sanothimi	*Pyxine cocoes*	5.94±2.49	4.72±1.82	0.08±0.23
8.	Pepsicola	*Caloplaca sp*	9.9±2.49	3.97±1.82	0.83±0.23
9.	Tripureshwor	*Hypotrachya revolute*	4.95±2.49	0.94±1.82	0.21±0.23
10.	Lagankhel	*Pyxine cocoes*	5.68±2.49	6.52±1.82	0.26±0.23
11.	Hattiban	*Pyxine cocoes*	6.27±2.49	5.29±1.82	0.78±0.23
12.	Trichandra College	*Pyxine cocoes*	7.67±2.49	5.27±1.82	0.27±0.23
13.	Gaurishankar	*Pyxine cocoes*	5.26±2.49	5.52±1.82	0.29±0.23
14.	Swayambhu	*Pyxine cocoes*	13.08±2.49	3.1±1.82	0.54±0.23
15.	Dallu	*Pyxine cocoes*	5.96±2.49	1.38±1.82	0.18±0.23
16.	Gaushala	*Pyxine cocoes*	6.97±2.49	3.78±1.82	0.06±0.23
17.	UN park	*Pyxine cocoes*	4.72±2.49	2.5±1.82	0.24±0.23

Heavy Metals Prevalence in Kathmandu Valley

Cadmium

Cadmium was observed in trace amounts only. The concentration of Cd was found highest at Pepsicola (0.83 µg/g) followed by Hattiban (0.78 µg/g) and Tilganga (0.69 µg/g) areas.

Mean: 0.379411765 µg/g

Standard deviation: 0.232041769 µg/g

Lead

The highest concentration of lead was found in the Swayambhu area (13.08 µg/g), whereas the lowest lead concentration was found in the Budhanilkantha area (2.45 µg/g).

Mean: 6.276470588 µg/g

Standard deviation: 2.490496791 µg/g

Chromium

The highest concentration of lead was observed in Lagankhel, Trichandra Campus Area, Hattiban areas, and the lowest was at TU road Kirtipur (0.46 µg/g).

Mean: 3.242352941 µg/g

Standard deviation: 1.823631437 µg/g

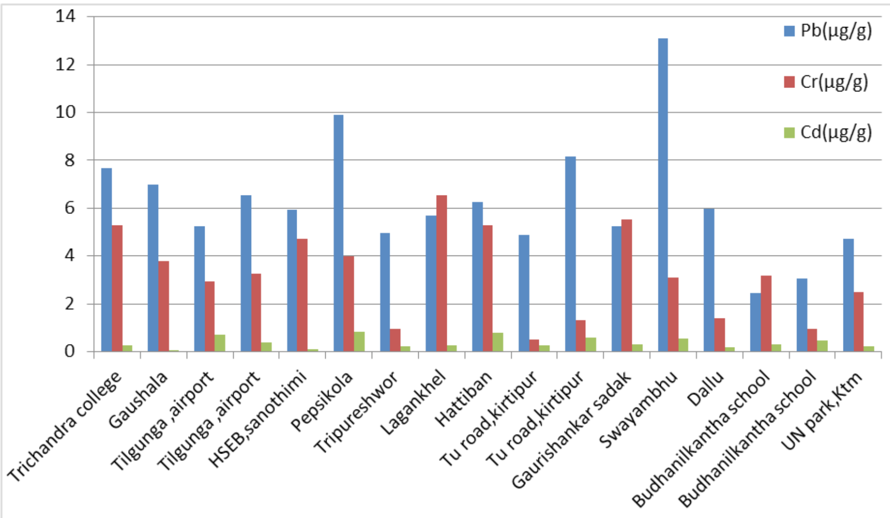

Fig. 2. Heavy metal distribution graph corresponding to sample ID.

The ANOVA test showed significant variation between the means at 5% level of significance.

4. DISCUSSION

A high concentration of Cd in the Pepsicola area would be connected to the farming area near the lichen collection site. The primary sources of Cd are fertilizer, mining, pesticide, plastic, refining, welding, etc. (Ayangbenro & Babalola 2017). Latter results also justified this finding. The highest concentration of lead could be the direct effect of exhaust emissions from petrol and diesel-driven vehicles. We found a significantly high lead concentration in samples obtained from major traffic junction areas like the Swayambhu, Pepsicola, and Tri-Chandra area of Kathmandu valley. Lead is a toxic heavy metal. A significant concentration of chromium observed in Lagankhel, Trichandra, Hattiban areas in the proximity of high vehicular flow may represent a high automobile industry near these areas. The primary user of Cr, primarily as an additive in unleaded gasoline, in recirculation cooling waters (antifreeze), as paints on automobile bodies, as trims and alloys on automotive parts, and in-car metal plating (Ward 1988). Comparatively lower concentrations of Cr at areas like Swayambhu and Pepsicola where Pb concentrations were high, could suggest an alternative source of Cr than vehicular emissions.

Our study produced similar conclusions to previous studies conducted by various groups in Kathmandu valley. Active and passive monitoring of lichens showed the highest concentration of heavy metals in high-traffic areas like Tripureshwor, Ratnapark, Bhadrakali Gaushala, and Kalanki. Passive monitoring indicated high Cd contamination in the Airport area (Chettri *et al*. 2015). In the study conducted around Hetauda industrial area (HIA) for Cr, Ni, Pb, Zn, Co, Mn, Fe, Si, and Al, higher levels of metals were recorded inside the industrial area compared to samples obtained from outside the industrial area (Pandey *et al*. 2002). In another study conducted to understand the level of heavy metals in ambient air of Kathmandu valley showed severe contamination of Pb, Cr, and Zn and relatively low contamination of Co, Cu, Mn, and Ni (Shakya *et al*. 2014).

Our study is also in accord with studies conducted around the world. In the Chinese city of Shijiazhuang, street dust heavy metal pollution sources were detected as traffic and industries (Cai & Li 2019). Twelve different metals in lichens thalli were studied in samples collected from Pichavaram mangroves of Tamil Nadu, India. The highest concentrations of heavy metals were exhibited by lichens near industrial areas and high vehicular activity (Logesh 2014). In a study conducted at different locations in Singapore, sites associated with heavy petroleum and shipping industries and road traffic showed peak concentrations (Ng, Tan, & Obbard 2006).

More and more people are buying automobiles, which are highly concentrated in developed city areas. Degradation in quality of air, land and water due to heavy metal along with other pollutant is serious. Our study, like other such studies performed before, place vehicular emissions and industrial waste as main cause for heavy metal pollution in Nepal. Managed urbanization planning and limiting vehicular concentration in specific areas should be implemented, if we are to tackle these problems.

5. CONCLUSION

From this study, it can be concluded that lichens are good bioindicators and can be used to reflect the heavy metal levels in our environment. Heavy metal pollution has become a major issue around the globe due to industrialization and fossil fuels. With increase in consumption of fossil fuel by vehicles and industries, heavy metal concentration in air has increased, which is reflected in lichen population. The heavy metals are found in high concentrations around areas with high traffic density like Tripureshwor, Kalanki, Pepsicola and so on. Exposure to such heavy metals is detrimental for human as well as animal health.

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