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Impact of Air Pollution on Growth of Lichens in Miandam Valley Swat, Pakistan

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ABSTRACT

This study was conducted to determine heavy metals *i.e.* Lead (Pb) and Cadmium (Cd) concentrations in lichen along different roads having varied traffic density and to find impact of the metals on its growth in Miandam valley Swat, Pakistan. *Dermatocarpon miniatum* species samples were collected from control site, primary, secondary and tertiary roads and were investigated for Pb and Cd concentrations and reduction in chlorophyll contents. The data were analyzed using ANOVA, correlation and regression analysis. Significant ($P<0.05$) variation in Pb and Cd concentrations in lichens along different roads were found. It was concluded that traffic density was responsible for Pb and Cd accumulation in lichen. Present findings further highlighted that there was significant variation in the chlorophyll content which amplify the heavy metal accumulation and subsequently inhibited growth of lichens.
Key words: Air pollution, Lead, Cadmium, Lichen, Roadside, Traffic

INTRODUCTION

Our flora has many highly fragile and sensitive biological species that can help in bio-monitoring of different pollutants generated from various sources. Among the sources transportation is one of the most notorious. With each passing day we need more and more vehicles to meet the demands of the increasing population, which leads to an increase in production of vehicular emissions. It is obvious these emissions are one of the main sources of air pollution. To determine pollutants the use of bio-monitors is necessary because the estimation of contaminants in atmosphere needs costly and specialized apparatus (Pignata *et al*., 2002). Llichens have high capacity to retain air contaminants. Moreover imperviousness to ecological issues and life span are qualities that make them most proper living beings for bio-monitoring studies (Garty, 2001; Wannaz *et al*., 2012; Paoli *et al*., 2011 and Garty, 2001). For environmental assessment the lichen bio-monitoring is an important tool, with abundance of applications in the assessment of most notorious contaminants, such as, heavy metals, radio nuclides, and nitrogen/sulphur oxides (Figueira *et al*., 2003). Several reports clearly indicated that the severe decline in many lichen species all over the world is because of air pollution (Nimis *et al*., 2001; Davies *et al*., 2007 and Larsen *et al*., 2007).

Vehicular emissions are considered as one of the main sources of air pollution. Heavy metals such as Pb and Cd accumulation due to traffic have been reported in various samples (Sesli, 2006; Dundar, 2006). The Pb is added to petrol as natural tetra-alkyl lead and ethyl-tri methyl lead. Engine autos discharge about 80 mg Pb per kilometer (Tuzen, 2003). The wearing of vehicular tires and carburetors releases Cd. In addition, the oil lubricant commonly used in vehicles has Cd, zinc and copper (Tuzen, 2003).

Both the heavy metals (Pb and Cd) are known for their toxicities. The presence of Pb in environment can cause numerous serious illnesses, including the neurological disorders and break down of renal blood cells (Smith, 1981). Cadmium accumulates mainly in kidneys, bones and lungs. Both high doze and long-term exposure can damage kidneys, while it can also affect bones due to average chronic exposure (Navas-Acien *et al*. 2004). In plants cell components, such as biological membranes can be damaged by the reactive oxygen produced by Pb accumulated in plant tissue (Sharma & Shanker, 2005). Furthermore, both Pb and Cd can influence enzymatic activities, degradation of chlorophyll contents and prevention of photosynthesis in plants (Fargasova, 2001, 2004; Haider *et al*., 2006). Road traffic is expected to remain a key contributor to air pollution in future (Truscott *et al*., 2005). Keeping in

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view the present increasing trend of vehicles in the study area the current study was designed to evaluate impact of air pollution on growth of lichens in Miandam valley Swat, Pakistan. The main objective of the study was to determine selected heavy metal concentrations in lichen collected from the primary, secondary, tertiary roadsides and control site of the area and to find impact of the metals on its growth.

MATERIAL AND METHODS

Study Area Description

The study area located in Swat (°34' to 35°55' N latitude 72°08' and 72°50' E longitudes) is a district of Malakand division, Khyber Pakhtunkhwa (KP), Pakistan. It occupies an area of 8220 km^2, with a population of 1.2 million (Census report, 1998). It is a habitat of an enormous array of flora and fauna, with significant number of widespread taxa and yet unknown species as well. Based on road size and traffic density, the roads can be divided into primary, secondary and tertiary. Similarly, there are different vehicles i.e. trucks, buses, motor cars, rickshaws etc consuming diesel, petrol and natural gas and their number is increasing with each passing day.

Lichen Sampling

A total of 24 samples of same species of lichens were collected along primary, secondary, tertiary roads and control site. All the samples were collected at a distance of 2 meter from each road. The samples from control site were collected at a greater distance from the general roads where it was assumed that negligible quantity of pollutants might have reached. Lichens were carefully collected from rocks with the aid of a stainless-steel knife at 1.5–2 m above the ground to avoid contamination from soil. The collected samples were neatly packed in polyethylene bags and transported to the laboratory for preparation and subsequent analysis (Boamponsem, 2010).

Heavy Metals Analysis

For heavy metals analysis, the lichen's samples were oven dried for 12 h at 90°C. 2 grams of each dried sample were grinded to powder with mortar. Then, 0.5 gram of each sample was put into a digestion tube. A mixture of concentrated HNO_3, $HClO_4$ and H_2SO_4(5:1:10) was added to each tube and left for 24 hours. After that, the tubes were adjusted in a digestion block at temperature 80°C for one hour and then at 120-130 °C for complete digestion. The digested solutions from the tubes were filtered through Whatman filter paper no. 42 into acid washed volumetric flasks and diluted to 50 ml with double distilled water. Finally, heavy metals (Pb and Cd) concentrations were determined by atomic absorption spectrometer (Analyst 700 Perk Elmer USA) (Khan *et al*., 2011).

Rocks Sampling and Analysis

A total of 24 rock samples were collected from the lichen's substrate rocks in neat and clean polyethene bags. The samples were grinded to powder. An amount of 0.5 grams was taken in a digested tube. Then 20 ml of Aqua regia was added and heated on a hot plate till dry. After that 20ml of normal HCL was added. It was heated again to a small volume, then cooled, after that the samples which were filtered into "acid washed volumetric flasks. The volume of the flask was adjusted to 50 ml by adding double ionized distilled water. The Pb and Cd concentrations in the digested solutions were measured using atomic absorption spectrometer (analys 700 Perk Elmer USA).

Chlorophyll Analysis

A 50 mg of samples of lichen thalli were directly submerged in 5ml dimethyl sulphoxide (DMSO). Extraction was carried out in absence of light for 40 min at 65°C (Ronen and Galun 1984). Extraction was completed, until a 'ghost like' colorless thallus appeared. The spectrophotometer UV-Unico-2100 Model was calibrated at zero absorbance using a blank of pure DMSO. At 645 and 663 nm absorbance of blank and samples were measured no longer than 20 minutes' procedure was completed after the extraction. A blank of pure DMSO was run and chlorophyll 'a' (Chl a), chlorophyll 'b' (Chl b) and total chlorophyll were calculated by following equation of Arnon's (1949)

"Chla (g/L) = 0.0127 A663 – 0.00269 A645" _____ (Eq.1)
"Chlb (g/L) = 0.0029 A663 – 0.00468 A645" ____ (Eq.2)
"Total Chl (g/L) = 0.0202 A663 + 0.00802 A645" _______ (Eq.3)

The values of absorbance at 663 nm and 645 nm wave lengths were put into each equation for calculation of the respective chlorophyll content present in lichens.

Statistical Analysis

The data were statistically analyzed by statistical packages SPSS 17. One-way analysis of variance (ANOVA) was used to verify the variability and validity of results. Linear regression analysis was carried out to check relationships between the heavy metal (Pb and Cd) concentrations in lichens and rocks and its relationship with chlorophyll content.

RESULTS AND DISCUSSIONS

Pb Concentration in Rocks

Table 1 shows the level of Pb in rocks of control site and different roads. The mean concentrations of Pb in rocks were 8, 3, 6 and 7 mg/kg of control site, primary, secondary, and tertiary road, respectively. The concentration of Pb was found higher at control site as compared to concentrations of Pb found in that of primary and secondary roads rocks. This variation in the Pb concentrations were due to the geological factors rather than traffic density (Koz *et al*., 2010). It was found from the Pb concentration in lichens (table I) that a significantly low concentration of Pb was found in lichens collected from control site due to lower traffic density.

Cd Concentration in Rocks

The concentration of Cd in rocks of control, primary, secondary and tertiary road were 4.9, 2.2, 2.3 and 5.3 mg/respectively (table I). The concentration of Cd in rocks of the tertiary road site was higher than other sampling points. The primary site had the lowest concentration of Cd. By comparing the results of Cd concentration of rocks with its concentration in lichens it was found that surface geological factors instead of traffic load were responsible for this variation in the rocks of control, primary, secondary and tertiary road.

Actual Concentration of Pb in Lichens

The actual mean concentrations of the Pb in lichens were 4, 5.8, 3.6 and 5 mg/kg of control site, primary, secondary and tertiary road samples, respectively (Table 1). The result of one-way ANOVA at $P \leq 0.05$ showed significant variation in the accumulation of Pb in lichens at selected roads and control site. This significant variation in the concentration of Pb accumulation in the lichens along different roads showed that traffic density was the main factor responsible for the variation as the study area was having no other gasoline or fuel burning units i.e. industries.

The presence of Pb in the study area's environment is a matter of concern because the toxicity of Pb is a well-known fact. Exposure to it may cause a number of fatal diseases. It was found that there is a strong linkage among human population, urbanization and Pb release in the environment. It can badly affect the human nervous system in general. However, children are main target in terms of Pb toxicity. Its concentration in human body severally damages kidneys that may lead to death (Meeeker *et al*., 2008).

Actual Cd Concentration in Lichen

The concentration of Cd in lichens of control, primary, secondary and tertiary road were 1.7, 2.2, 2.2 and 1.9 mg/kg, respectively (table 1). The results showed same concentration of the Cd found in primary and secondary road samples but different from the control site and tertiary road samples. This higher concentration of the Cd along tertiary road might be due to car washing points. As studies suggest that the release of Cd into the environment may occur because of combustion products and cleaning of carburetors (Bajpai & Upreti, 2012). The concentration of Cd reported in our study was higher than the reported values i.e. 0.24-1.4 mg/kg (Mendil *et al*., 2005), 0.191 mg/kg (Loppi *et al*.,1999), 0.26-2.08 mg/kg (Loppi *et al*., 2000), 0.047-0.162 mg/kg (Riget *et al*.,2000), accept the values i.e. 4.03 mg/kg in *Peltigeramembranacea* and 4.6 mg/kg in *Xanthoparme liaconspersa* (Uluozlu,2007) which were found higher than our reported values. The higher value of Cd in the study area suggested that higher concentration of Cd contamination in the environment was present as compared to the above mentioned studies.

Projected Pb Concentration in Lichen

The projected concentrations of the Pb in rocks were 4, 1.5, 3.1 and 2 mg/kg in control site, primary, secondary and tertiary road, respectively. Significant variation in the projected concentrations of Pb in lichens were found along with different roads. Projected concentration of Pb was calculated by assuming that a fraction of the Pb had been transferred from rocks to the lichen body. It was done to highlight the traffic factor rather than accumulation of the Pb from the substrate rock in lichen under this study (table I).

Table I: Heavy metal concentrations (mg/kg) in rocks and lichen and chlorophyll contents (g/l) in lichen

		Rock	Lichen actual	Lichen projected	Traffic Factor	Chla	Chlb	Total Chl
Control	Pb	8.00	4.05	4.05	N/A	0.0188	0.0031	0.152
	Cd	4.83	1.77	1.77	N/A			
Primary	Pb	3.01	5.80	1.52	3.55	0.0181	0.0028	0.148
	Cd	2.49	2.26	0.91	1.34			
Secondary	Pb	6.30	3.58	3.19	0.66	0.0185	0.0018	0.142
	Cd	2.59	2.25	0.95	1.30			
Tertiary	Pb	7.18	4.94	2.02	1.30	0.0182	0.0019	0.146
	Cd	5.73	2.00	2.10	0.10			

Regression Analysis

In order to find dependency of dependent variable (Pb concentration in lichens) on independent variable (Pb concentration in rocks) regression analysis was done. The R^2 values for the actual and projected Pb concentrations in lichen in primary, secondary and tertiary roads and substrate rocks are summarized in table II. The analysis showed that there was quite weak dependency of the actual Pb concentrations in lichens on substrate rocks while high dependency of the projected Pb concentrations in lichens was observed. From the table II it can be easily concluded that actual concentration of the Pb in lichens along different roads was quite poorly dependent on substrate rocks.

Projected Cd Concentration in Lichen

The concentration of Cd in rocks of control, primary, secondary and tertiary road sites are 1.7, 0.9, 1 and 2 mg/kg respectively.

The concentration of Cd in lichens of the tertiary road site was higher than other sampling points. The primary road site has the lowest concentration of Cd in lichens (Table I).

Regression Analysis of Cd

The R^2 value for the actual Cd concentrations in lichen on primary road and substrate rock was 0.041 that showed somewhat lower dependency of dependent variable on the in-dependent one. While the regression analysis of Cd concentration in rocks against the projected Cd concentrations in lichens showed a very strong dependency of $R^2 = 1$. the relationship was similar for the rest of the roads as given in Table II.

Traffic Factor

Impacts of Traffic on Pb Concentration in Lichens

The traffic impact on Pb concentration in lichens was found higher on primary road, lower on tertiary road and lowest on secondary road (Fig. 1). The reason of the high Pb concentration in collected lichens of primary road was due to intense traffic. In secondary road site, the Pb concentration in lichens was lower than primary road due to less traffic on secondary road. However, lichen collected near tertiary road had high Pb concentration due to congestion in the area

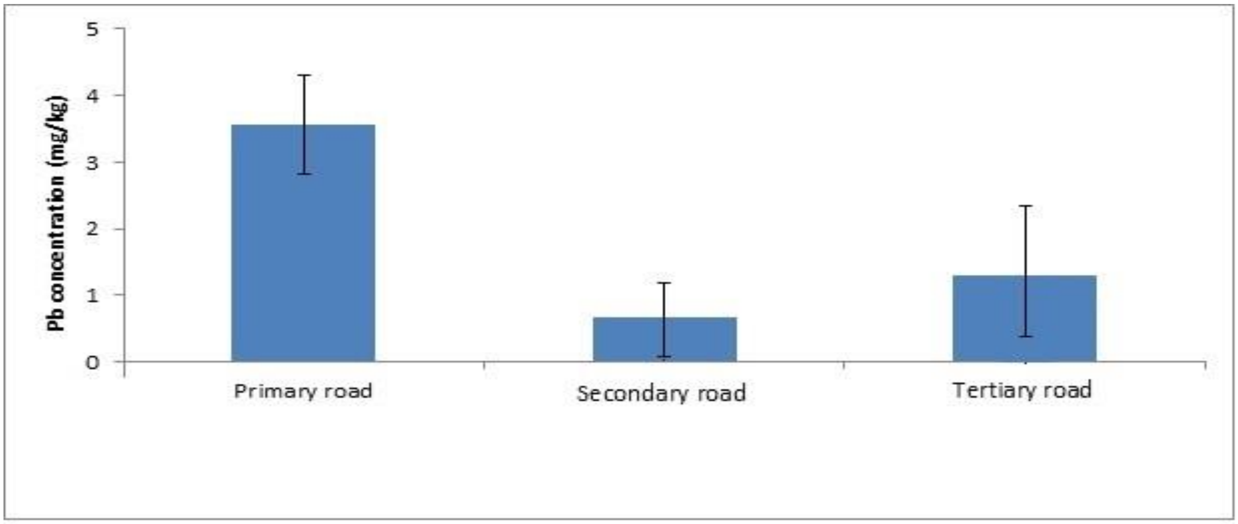

Fig. 1: Comparison of mean concentrations of Pb in lichen among primary, secondary and tertiary roadside sites: error bars indicate standard deviation.

The accumulation of Pb in lichens from all the possible sources is lower in Fig. 1. After comparing the overall contribution of Pb to the lichen body it was found that traffic was the main contributor of Pb in the environment on primary road.

Comparative analysis of secondary road was done in the same way. The contribution of traffic as main factor is highlighted in Table 1. The traffic that was responsible for Pb accumulation in lichen was found low an secondary road. This might be due the lower traffic density on secondary road as compared to the primary road (fig., 2).

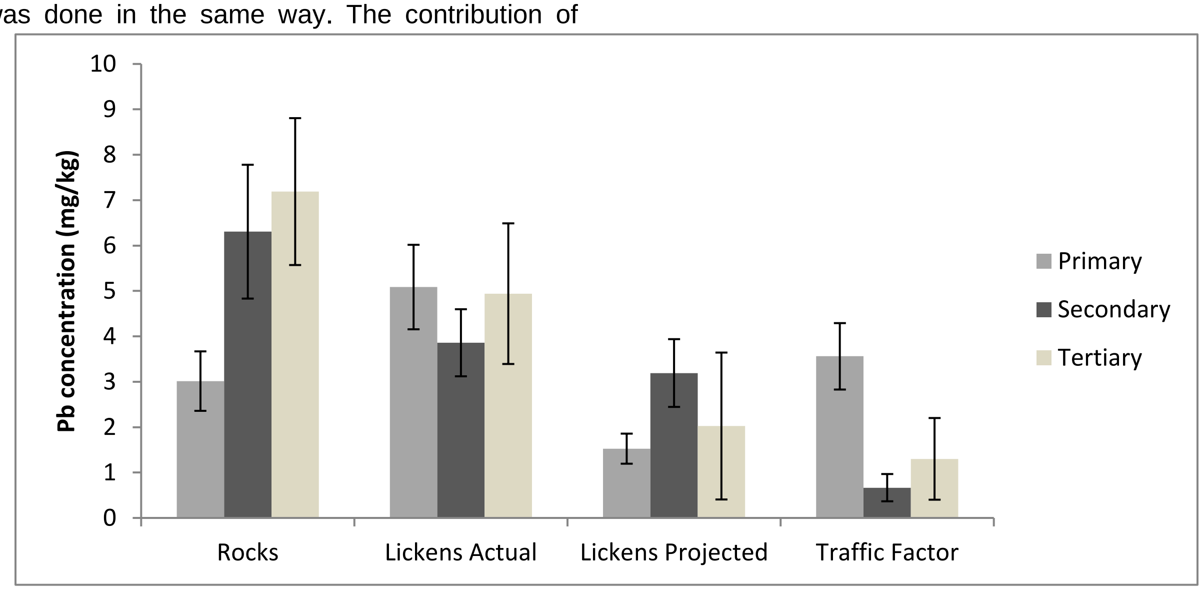

Fig. 2: Comparison of mean concentrations of Pb along primary, secondary and tertiary road sites: error bars indicate standard deviation.

The comparison method was repeated for the tertiary road as well as shown in Fig., 2. The concentration of Pb at tertiary road was found higher as compared to the secondary road. It is evident that although traffic density on tertiary road was quite low but still the concentration of Pb found on the tertiary road was higher. This higher concentration of Pb might be due to congested and wind still situation on tertiary road. As the dispersion effect of wind has also been reported (Garty *et al*., 2003 and Cloquet *et al*., 2006).

Impacts of Traffic on Cd Concentration in Lichens

The traffic impact on Cd concentration in lichens was found higher on primary and secondary road site and lowest on tertiary road. The reason of high Cd concentration in collected lichen of primary road was intense traffic. In case of secondary road site, the concentration in lichens was lower than primary road due to less traffic. However, the lichens collected near tertiary road had lowest Cd concentration due to less traffic (fig. 3).

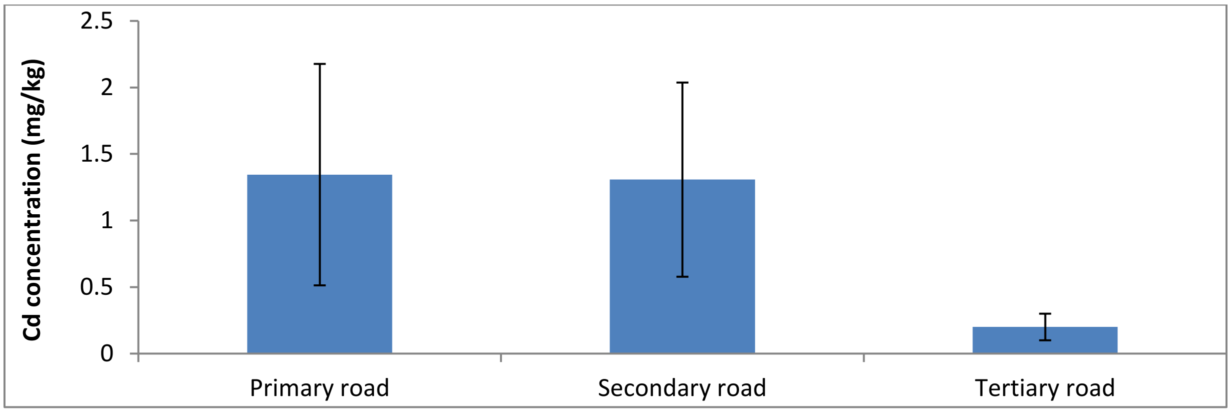

Fig. 3: Comparison of mean concentrations of Cd in lichen among primary, secondary and tertiary roadside site: error bars indicate standard deviation.

Apart from overall impact of traffic on Cd concentration in lichen, individual effect at primary, secondary and tertiary roads is also shown in Fig., 4. Traffic impact shown on primary road was found somewhat similar to that of secondary road. Thus higher concentration of Cd at secondary road might be due to car washing points. Previous studies have reported that Cd is used in carburetor and other parts of cars. The metal might be released into the environment through wear and tear and servicing of vehicles (Tuzen, 2003; Narin and Soylak, 1999). As shown in the figure lower concentration of Cd was found at tertiary road that further provided the evidence that lower traffic density on this road might be the reason of lower metal concentration.

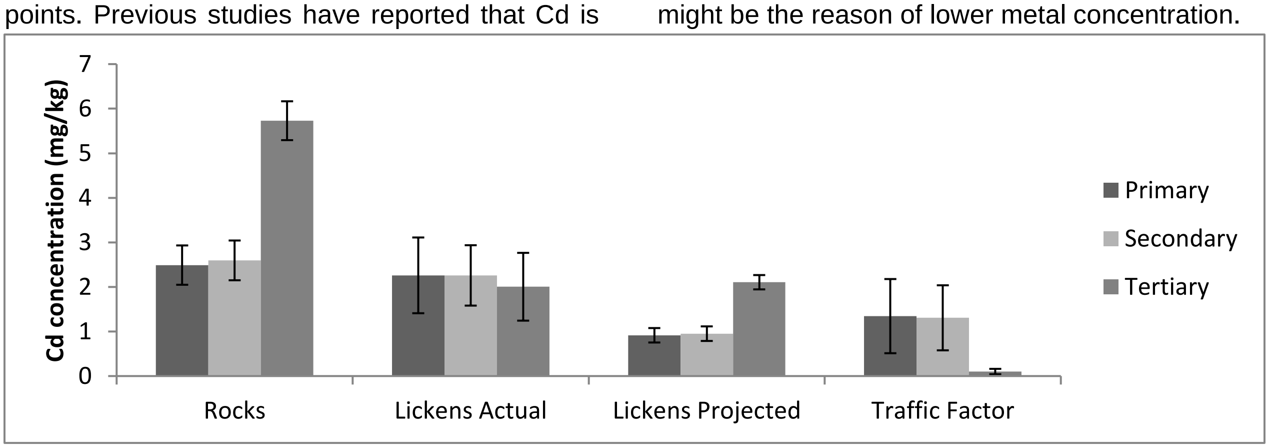

Fig., 4: Comparison of mean concentrations of Cd along with primary, secondary and tertiary road sites: error bars indicate standard deviation

Impacts of Traffic on Chlorophyll in Lichen

The total chlorophyll concentrations in lichen were 8, 3, 6.3 and 7 at control, primary, recently (g/l) Cd tertiary road site respectively. High concentration of total chlorophyll was found in lichen of control site. Tertiary road had high concentration of total chlorophyll in lichen as compared to the primary and secondary road. The primary road had the lowest total chlorophyll concentration in lichen (Fig.,5).

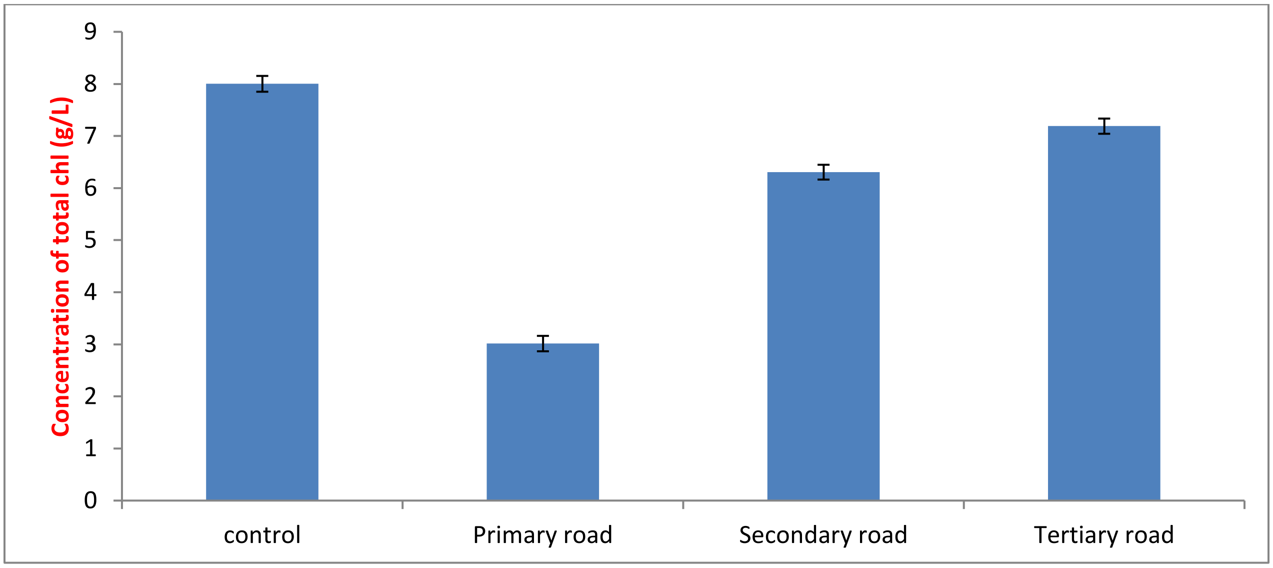

Fig. 5: Comparison of total chlorophyll concentration (g/L) in lichens of various sampling points**.**

It is recognized that heavy metals interfere with chlorophyll synthesis by means of induced deficiency of one of the most important nutrient or through dysfunction of enzymatic activity. An inverse correlation has been found in the chlorophyll content and the presence of heavy metals in the thallus of lichen (Yildiz *et al*., 2011 and Garty, 2001).

In addition, Cd inhibits overall growth by attacking and changing metabolic activity of lichen. It may cause changes in the photosynthesis promoting protein, metabolism, stress response and cell structure (Xie *et al*., 2014). The Cd action alters metabolic enzymes, including ATP synthases sub units and plenty of photo system components (Alessandro *et al*., 2013). These malfunction may lead to growth retardation in plants that results in lower production and yield.

Thus, the aforementioned results of our study showed that both Pb and Cd were responsible for the degradation of chlorophyll content in lichens.

Regression Analysis of Chlorophyll and Pb Concentration

Regression analysis of Pb and Cd concentrations in lichen and its relationship with chl a., chl b and total chlorophyll (total chl) were determined (Table II). The R^2 values for the Pb concentrations in lichens on primary road and chl a was 0.787 that showed high dependency of chl a on the Pb concentrations in lichens. While the regression analysis of the Pb concentrations and Chl b also showed dependency at R^2 =0.987 as shown (Table II).

Regression Analysis of Chlorophyll and Cd Concentration

The regression analysis of concentration of Cd and degradation of chlorophyll was done as shown in (table II). For chl a degradation and the concentration of Cd in lichens the R^2 value was 0.327. The value showed mild dependency of chl a on the concentration of Cd. It means that by increasing Cd concentration in lichen, the content of chl a would be reduced. In the same way the regression model of chl b and Cd concentration was found for which the R^2 value was calculated as 0.015 that indicated quite lower dependency of chl b on Cd concentration as shown in (Table II). The lower R^2 value suggested that by increasing the Cd concentration in lichens no significant change in the content of chl b would occur (table II)

Table II: Linear regressions model for heavy metal concentrations in rocks and lichen and lichens chlorophyll contents

Treatments	Heavy metals	R^2	
		Heavy metals rocks to lichen	**Heavy metals concentration and chl contents**
Primary	Pb	0.5098	0.7876
	Cd	0.0145	0.3271
Secondary	Pb	0.0115	0.9879
	Cd	0.0444	0.0155
Tertiary	Pb	0.5486	0.324
	Cd	0.2194	0.5958

Conclusions and Recommendations

This research provides primary data for metal concentrations at various road sites which can provide useful information for future biomonitoring research studies in the vicinity. The contents of Chlorophyll were a proper measure to investigate the aerial contamination of an area. The physiology of lichens was significantly affected by the heavy metals under research Pb and Cd. The most appropriate indicator for advance biomonitoring is the sensitivity of photosynthetic parameter with changeable levels of heavy metals. The presence of Pb and Cd in lichen samples is a clear indicator that a significant quantity of the heavy metals is released in the study area through vehicular exhaust. There is a dire need to ensure lead-free gasoline in the study area to save the environment from toxic effects.

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