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Evaluation of urban air pollution by metal contents of woody vegetation leaves in the urban ecosystem

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Abstract

Urban air pollution is a major environmental concern, and it should be addressed on a priority basis for human health and the urban ecosystem. The study was performed to investigate and understand the spatial distribution and contamination levels in the leaves of selected plants (*Eugenia jambolana, Morus alba*, *Dalbergia sissoo, Populus deltoides, Ficus religiosa, Ficus variegata, Cassia fistula, Eucalyptus camaldulensis, Melia azedarach, Psidium guajava, Pongamia pinnata, Callistemon citrinus,* and *Polyalthia longifolia*) exposed to the polluted areas of Canal Road, Lahore. Metal concentrations (Pb, As, Cr, and Cd) in the leaves of the plants were analyzed using atomic absorption spectrometry (AAS). The contamination level of As (Average ~1.03 mg/kg) was found to be moderately low in all trees tested except in *Eucalyptus camaldulensis* (As~2.11 mg/kg). Lead (Pb) accumulation levels in the leaves were observed to be visibly higher in almost all samples (Average ~ 5.34 mg/kg) than the WHO recommended limit (2 mg/kg). Among all samples, *Ficus religiosa* was found to have the highest levels of Pb. The contamination trends of Cr were observed to be high (Average~1.06 mg/kg) in non-native species, specifically in *Eucalyptus camaldulensis* (3.21 mg/kg). The Cd concentrations were also found to be higher in all plant samples (Average ~1.90 mg/kg) than the WHO permissible limit (0.02 mg/kg) in plants. Principal Component Analysis (PCA), GIS, and Minitab-19 were also applied to the data. This work is important to set a baseline for future researchers to appraise pollution load in different areas in light of the findings of this study.

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Introduction

The atmosphere, a multifaceted and dynamic gaseous matrix, stands as a critical entity responsible for upholding and nurturing life on our beloved planet, Earth. While encompassing an intricate amalgamation of compounds, this atmospheric framework performs an array of indispensable functions (Sarmiento-Cano et al., 2022). However, the undeniable reality persists those human activities in the form of anthropogenic endeavours, possess the potential to significantly impinge upon the delicate equilibrium of our environment. Among the plethora of concerns that arise from our actions, atmospheric pollution emerges as a ubiquitous predicament demanding immediate attention (Ashraf et al., 2010; Seager et al*.,* 2012; Manisalidis et al., 2020).

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The influence of different meteorological conditions such as sunlight, and particulate and gaseous air discharge react to produce a number of toxic pollutants that are dangerous for humans, animals, and plants (Wu et al., 2024). Heavy metal pollution is the most prominent component in atmospheric pollutants that are mainly caused by immobile or mobile pollution sources, such as power generation services, waste incineration, domestic oil burning, vehicular traffic, industrial activities, or construction and demolition activities (Aziz and Bajwa, 2007; Das et al., 2023). Once these pollutant particles enter the atmosphere they can move easily with wind. Pollution and emission level variations depend on the town planning and infrastructure because buildings are also counted as pollution emission sources due to various power-consuming activities (Manisalidis et al*.* 2020).

Pollution can affect human health in numerous ways; several factors influence human health, e.g., composition of air pollutants, time of exposure, and dose (Tran et al., 2023). Pollution exposure in humans can cause difficulty in breathing, nausea, cancer, skin irritation, birth defects, reduced activity of the immune system, and developmental delay in children (Santos et al., 2019). The most common heavy metal air pollutants are mercury, cadmium, and lead (Mitra et al., 2022; Naz et al., 2023), impacting adversely the environment.

Urban areas' air pollution components due to a significant rise in air pollution emissions brought on by fast economic and industrial development over the past century, air quality has become a significant global environmental issue (Rehman, 2018). In cities, particulate matter, ozone, and nitrogen dioxide are the most prevalent pollutants in the air (Ritz et al., 2019). Particularly, prevalent in industrial locations is sulphu dioxide. Fungal spores or plant-derived particles (pollen grains and tiny elements, such as soybean dust and *Ricinus*) carry and distribute aeroallergens (D'Amato et al., 2000, D'Amato et al., 2002, D'Amato et al., 2005, Annesi-Maesano et al., 2007; D'Ovidio et al., 2023). According to the United States Environmental Protection Agency, it is estimated that more than 50% of Americans reside in locations where ozone, nitrogen dioxide, sulphur dioxide, and particulate matter levels are higher than the current national ambient quality standards (Dockery and Stone, 2007).

The objective of the present study was to determine the concentrations of selected metals in the leaves of some woody species in an urban ecosystem so as to identify the pollution-sensitive species as bio-indicators of pollution and understand their spatial distribution pattern.

Materials and Methods

Study area

A comprehensive study was conducted involving the collection of plant samples from different locations along the Canal bank within Lahore City (**Figure 1**). Specifically, the selected sampling sites involved the most busy and congested regions within the city, which stretch from Thokar Niyaz Baig to Jail Road within the Lahore city. It was within this carefully described area that a variety of plant leaves were systematically and randomly collected from a diverse range of plant species. The study sought to explain the influence of heavy traffic on various parts of the atmospheric composition. This intricate examination intended to unravel the complex relations between vehicular emissions, plant biology, and the overall dynamics of the surrounding environment.

Figrue 1. Description of sampling site through a map (Canal road)

Lawrence Garden (Bagh-e-Jinnah) (**Figure 2**) is a large historic park in Lahore with a zoo, open-air theater, library, sports facilities, and about a 2 km jogging track. This park is roughly comprised of about 140 types of shrubs, 150 types of trees, 50 varieties of creepers, 30 palms, and huge types of annual flowers. The annual flower show is held every spring season, and the worth visiting occasion in Lawrence Garden is the annual *Chrysanthemum* show (Qasim, 2015).

Figure 2: Description of sampling site through the map (Lawrence Garden).

Selection of plant species

Thirteen common plant species were chosen for this study, comprising a variety of local and non-native species. All of these plants are abundantly planted along the bank of the Lahore canal or at green belts on the canal road, and the leaves were sampled using a random selection approach. Some fruiting plants, such as Java plum, white mulberry, and guava, were chosen for heavy metal analysis due to public health concerns, as were some species with economic or beneficial significance, and some decorative species.

These species were *Eugenia jambolana* (Java plum or jamun), *Morus alba* (White mulberry), *Dalbergia sissoo* (Indian rosewood or Shesham), *Populus deltoides* (Eastern cottonwood) *Ficus religiosa* (Sacred fig or peepal) *Ficus variegata (*Fig tree*), Cassia fistula* (Amaltas)*, Eucalyptus camaldulensis* (Gum tree/safeda)*, Melia azedarach* (White cedar/Drek)*, Psidium guajava* (Guava/Amrood), *Pongamia pinnata* (Indian beach tree/Sukh Chain), *Callistemon citrinus* (Bottle brush) and *Polyalthia longifolia* (False ashoka/Ulta sauk).

Sampling of plant leaves

Leaves of all selected species were collected from different sampling sites selected for this study along the Lahore Canal. Two leaves of each plant were sampled from each site. Leaf area with the help of a scale was also noted for every single species. The collected leaves were fully expanded young. The leaf samples were made free from dust and kept in a metal-free white paper.

Sample analysis

Dried and processed plant samples (each 0.5 g) were taken into each of MF/HF vessels of a microwave oven. Three mL of concentrated HNO_3 were added to the sample (in the fume hood), and carefully spun, then 3 mL of concentrated H_2O_2 and 0.5 mL of concentrated HCl were added to each vessel. The vessels were placed in a microwave the temperature of which was set at 100 °C for 10 minutes, then allowed the samples to cool down. The metals such as Pb, As, Cr, and Cd were determined using an atomic absorption spectrophotometer.

Statistical analysis

Statistical analysis was done to estimate the concentration trends of heavy metals in the selected trees. Biomass of all trees was calculated using the tree biomass estimation formula (MT ~ exp (-2.977+in ρD2HT)) where Mt (kg) is dry mass above ground, D (cm) diameter, HT (cm) height, and ρ (g/cm^3) is the wood density. The average values of every parameter were also drawn by using the MS Excel average formula. Minitab 19 was used to generate PCA or also to calculate dependencies of different parameters and their clustering trends; GIS was also used to generate spatial distribution maps of vast data.

Results and Discussion

Field data obtained during the sampling is given in **Table 1**. In this study, we analyze the vehicular traffic impacts on roadside vegetation as well as on the environment by applying heavy metal detection tests on leaves of different tree species. The leaves of different plant species planted along the Canal bank or at Canal Road and 5 leaf samples from the Lawrence Garden (as control), were analyzed in this study. All field observations of 18 different sites and field or lab data were collected and tabulated. The analysis shows that all sites are considered to be heavily polluted with Pb, but the concentrations of As, Cr, and Cd are different in samples from different sites. This species examination shows that due to heavy traffic and the industrial sector, most of the sites of Lahore are affected by pollution.

Table 1. Field data including longitude, latitude, tree height, and diameter, carbon mass, tree canopy, leaf arrangement, and other parameters

ID	Lon	Lat	PH	LEA	Dia	CM	TCan	LAr	Leaf Condition
LTN1	74.35479	31.47125	4.27	43.9	0.182	0.27	3.05	Alternate	Dark green/Healthy, disease free/Mature
LTC2	74.31527	31.50776	9.14	40.6	0.488	3.76	7.62	Alternate	Medium green/Healthy, disease free/Mature
LTZ3	74.35479	31.55002	11.28	86.1	0.731	10.48	6.10	Alternate	Dark green/Healthy, disease free/Mature
LTJ4	74.34137	31.53601	9.45	62.3	0.518	4.58	7.01	Opposite	Glossy dark green/Healthy, disease free/Mature
LTR5	74.27798	31.45642	8.23	102.0	0.549	4.44	3.96	Opposite	Light green/Healthy, disease free/Mature
LLG6	74.33047	31.55327	12.50	86.1	0.762	13.11	5.49	Alternate	Dark green/Healthy, disease free/Mature
LLG7	74.33047	31.55327	4.57	102.0	0.213	0.41	1.83	Opposite	Light green/Healthy, disease free/Mature
LLG8	74.33047	31.55327	7.62	43.9	0.579	4.49	7.92	Alternate	Dark green/Healthy, disease free/Mature
LLG9	74.33047	31.55327	8.84	62.3	0.579	5.03	5.79	Opposite	Glossy dark green/Healthy, disease free/Mature
LLG10	74.33047	31.55327	11.89	40.6	0.640	8.75	7.01	Alternate	Medium green/Healthy, disease free/Mature
LTQ11	74.29901	31.49056	4.57	20.9	0.305	1.18	3.05	Alternate	Glossy dark green/Healthy, disease free/Mature
LTP12	74.25317	31.4738	6.10	51.6	0.762	3.58	5.49	Alternate	Smooth and light green/Healthy/Mature
LTB13	74.31905	31.51188	12.19	23.8	0.366	4.99	2.44	Alternate	Bluish green/Healthy/disease free/Mature
LTS14	74.23914	31.46908	7.62	20.8	0.305	4.49	6.10	Bi-pinnate	Dark green/Healthy, disease free/Mature
LTG15	74.32248	31.51546	4.57	52.7	0.792	1.09	2.44	Opposite	Green to brownish green/May be leaf curling syndrome/Mature
LTH16	74.27703	31.47977	5.49	61.9	0.945	1.59	3.35	Alternate	Glossy deep green/Healthy, disease free/Mature
LTF17	74.33458	31.52819	6.40	1.9	0.030	1.69	4.88	Alternate	Dark green with small soft hairs/Healthy, disease free/Mature
LTU18	74.32561	31.51901	8.23	39.7	0.610	2.89	2.74	Alternate	Dark to lime green/Healthy, disease free/Mature

LON = Longitude; LAT; Latitude, PH= Plant height (m); LEA = Leaf area, Dia = Diameter (m); CM = Carbon mass (kg); TCan = Tree canopy (m); LAr = Leaf arrangement; LC = Leaf Color/Health/Stage

Traffic flow

The traffic flow was also considered to obtain useful information associated with the intensity of the vehicular flow. The variation of vehicular flow was estimated through direct manual observation for two days during three time periods in a day from 8:00 a.m. to 10:00 a.m. (morning max flow), 12:00 p.m. to 2:00 p.m. (afternoon max flow), and 5:00 p.m. to 9:00 p.m. (evening max flow).

Permissible limits by WHO

The World Health Organization (WHO) has established stringent permissible limits for heavy metal concentrations in tree leaves due to their potential environmental and health implications (**Table 2**). When heavy metal levels exceed these specific limits, they can lead to toxicity in plants, and if consumed by humans, they can pose health risks. Additionally, heavy metal accumulation in plant leaves can disrupt food synthesis pathways and interfere with environmental cleansing processes.

Table 2. WHO permissible limits of heavy metal (Cd, Cr, As & Pb) in plants (WHO, 1996).

Heavy metal	Permissible value of plants (mg/kg)
Cd	0.02
Cr	1.30
Pb	2.00

Metal concentrations in tree leaves

The findings show that lead (Pb) concentrations in the tree leaves from both the study and control sites exhibited a wide range of values, spanning from 0.6 mg/kg to 10.3 mg/kg (**Table 1**). In this context, it is important to note that the permissible limit for lead (Pb) in plants, as set by the World Health Organization (WHO, 1996) is 2 mg/kg of dry weight. The high concentration of Pb was observed in LLG7, LTZ3, and LTJ4 (**Table 1**), which might have been due to the heavy traffic even at the control site because the samples were taken from road-facing side.

Lead is a component of older gasoline formulations and vehicles that use such fuels and can release lead into the atmosphere as part of their emissions (Bhasin et al., 2023). This can result in lead deposition on nearby surfaces, including the leaves of trees and plants (Collin et al., 2022). This study indicates that the more trees near a road have more exposure to vehicular traffic (cars, bikes, and heavy traffic) which might have resulted in high Pb concentration in leaves. On the other hand, LLG10 has a very low Pb concentration (Pb~0.6 mg/kg); the reason could be that these samples were taken from the control site with less traffic exposure. So, the Pb accumulation in leaves may be directly related to traffic size.

According to the British Herbal Medicine Association (1998), the normal range of arsenic (As) in plants is 5 mg/kg. However, arsenic cannot accumulate up to toxic levels in plants, but still has the ability to cause negative impacts on plants, animals, and humans. **Table 1** shows that arsenic (As) values range from 0.05 mg/kg to 2.11 mg/kg. The highest As concentration was recorded in LTB13 as 2.11 mg/kg and in LTP12 as 2.03 mg/kg, while the lowest values of As were recorded in LLG10 and LLG6 as 0.05 mg/kg. Generally, As level was low in those samples that were taken from off-road area, while the samples taken from the canal road displayed higher As concentration. The reason for high As concentration in the off-road samples might have been due to the mixing of polluted water with canal water or it might have come from vehicular exhaust, ultimately accumulating in the leaves through roots or through the air (Deng et al., 2024).

Table 1 also shows the concentrations of chromium (Cr) in the leaves of the trees along the Canal road and Lawrence Garden. Chromium concentrations in the plant samples ranged from 0.04 mg/kg to 3.21 mg/kg. The permissible limit for Cr recommended by WHO for plants is 1.30 mg/kg. Chromium concentration in some plant samples like LTB13 had the highest level of Cr as 3.21 mg/kg; LTP12 also had the high concentration of Cr as 3.02 mg/kg. On the other hand, LTJ4 had the lowest concentration of Cr (0.04 g/kg). These results indicate that vehicular traffic size also affected the leaf Cr concentration. The sampling sites with less traffic exposure showed low Cr concentration, while the trees at the sites with more traffic exposure had high Cr. The concentration of chromium (Cr) in plant leaves is influenced by a combination of human activities and natural processes. Chromium enters the environment through various sources such as industrial emissions and natural weathering of rocks, and its uptake by plants through roots that is typically low (Hussain et al., 2006; Prasad et al., 2021).

Cadmium (Cd) in the leaf samples was highly variable ranging from 0.7 to 6.1 mg/kg (**Table 1**). WHO recommended the permissible limit for Cd concentration in plants as 0.02 mg/kg. Thus, Cd levels in almost all leaf samples were higher than the recommended limit by WHO. Higher Cd concentrations were recorded in LLG9 (6.1 mg/kg), LTZ3 (5.71 mg/kg), and LLG6 (3.5 mg/kg). In contrast, lower Cd concentrations were found in LTR5 (0.7 mg/kg), LTN1 (0.848 mg/kg) and LLG10 (0.9 mg/kg) (**Table 1**). The reason for the high Cd concentration may have been the use of fertilizers and pesticide sprays for better plant growth in the Lawrence Garden, because Cd concentration was comparatively high in the control samples as compared to those of the samples from the Canal road. In fact, there was no major chemical industry or use of pesticides along the Canal road, so, there is no direct connection between vehicular traffic and Cd concentration, and Cd can enter in the atmosphere from a source other than vehicular traffic (Genchi et al., 2020).

Metal concentrations and species origin

The study data was also analyzed to establish if the origin of selected species (native or non-native) affected the accumulation levels of heavy metals and species belonging to which origin contained more contaminations. Generally, the accumulation of different metals was higher in the native species than that in the non-native ones (**Table 3**).

Table 3. Metal concentrations in native and exotic plant species

Sample ID	Species name	Origin	Pb (mg/kg)	As (mg/kg)	Cr (mg/kg)	Cd (mg/kg)
LTN1	*Morus alba*	Native	9.43	0.78	0.163	0.848
LTC2	*Dalbergia sissoo*	Native	5.33	0.5	0.047	1.66
LTZ3	*Populus deltoides*	Native	9.1	0.23	0.2	5.71
LTJ4	*Eugenia jambolana*	Native	8.3	0.25	0.04	1.8
LTR5	*Ficus religiosa*	Native	6.3	0.17	0.12	0.7
LLG6	*Populus deltoides*	Native	2.3	0.05	0.15	3.5
LLG7	*Ficus religiosa*	Native	10.3	0.71	0.3	1.1
LLG8	*Morus alba*	Native	0.7	0.2	0.58	1.8
LLG9	*Eugenia jambolana*	Native	6.9	1.88	1.05	6.1
LLG10	*Dalbergia sissoo*	Native	0.6	0.05	0.57	0.9
LTQ11	*Ficus variegata*	Non-native (Bangkok)	2.91	1.12	0.98	0.99
LTP12	*Cassia fistula*	Indian subcontinent	6.67	2.03	3.02	1.51
LTB13	*Eucalyptus camaldulensis*	Non-native (Australia)	6.45	2.11	3.21	1.67
LTS14	*Melia azedarach*	Non-native (Australia)	4.56	1.95	2.91	1.34
LTG15	*Psidium guajava*	Non-native (Australia)	2.86	1.76	0.32	1.45
LTH16	*Pongamia pinnata*	Native	5.21	1.99	2.34	1.23
LTF17	*Callistemon citrinus*	Non-native (Australia)	4.38	1.53	1.96	1.04
LTU18	*Polyalthia longifolia*	Non-native (India)	3.98	1.34	1.23	0.91
Higher concentrations trend			Mix trend	Non-native	Non-native	Native

Local species (Native) Exotic species (Non-native)

Dependencies on different growth attributes

In order to indicate the absence or presence of a direct relation between heavy metal content in leaves and the physical parameters of plants, scatter plot graphs were also designed for better explanation and to find correlation among different parameters. **Figure 3a**, **b**, **c**, **d**, and **e** explain the correlation and dependencies trends of different parameters that we analyzed during this study. **Figure 3a** represents the weak relation between As and Cd, as their correlation value had been noted as R^2~0.003, which represents the very weak correlation.

In **Figure 3b**, dependencies between Cd and C mass were noted to some extent; both are directly proportional to each other. Cadmium concentration tended to increase in plants with higher carbon mass. So, plants with more carbon mass are expected to have been exposed to more Cd pollutants. Their correlation value was R^2~0.3244 which is a comparatively strong correlation as compared to that between As and Cd.

In **Figure 3c**, dependencies of Cr concentration on the concentration of As show that both parameters have a comparatively strong correlation with a value of R^2~0.6632, which means in case of high As accumulation, Cr level must also be high. In **Figure 3d** the connection between the tree height and Pb level in leaves was measured which indicates that the level of Pb in plant leaves was not dependent on tree height; it may be low in tall trees and high in small trees. So, there must be some other reasons behind the high Pb level in leaves.

In **Figure 3e** dependency of Cr accumulation in leaves on leaf area basis is given, which indicates the inverse relation between Cr concentration and leaf area. For example, Cr level was high in trees with small leaves or low in trees with broader leaves. This may have been due to the effect of heavy metal contaminations on reducing leaf size. Correlation between them was also weak with a value of R^2~0.2731.

The number of anthropogenic or environmental factors that affect the metal concentrations in the environment or also in living components of the ecosystem, the climate of the area where trees are planted, and origin of species, are considered the environmental factors on which heavy metal accumulation depends (Sytar at al., 2020). Population distribution trends also have some prominent relation with pollution and pollutant availability in the atmosphere. The composition of atmospheric components is another reason for leaf contamination (Ossola and Farmer, 2024).

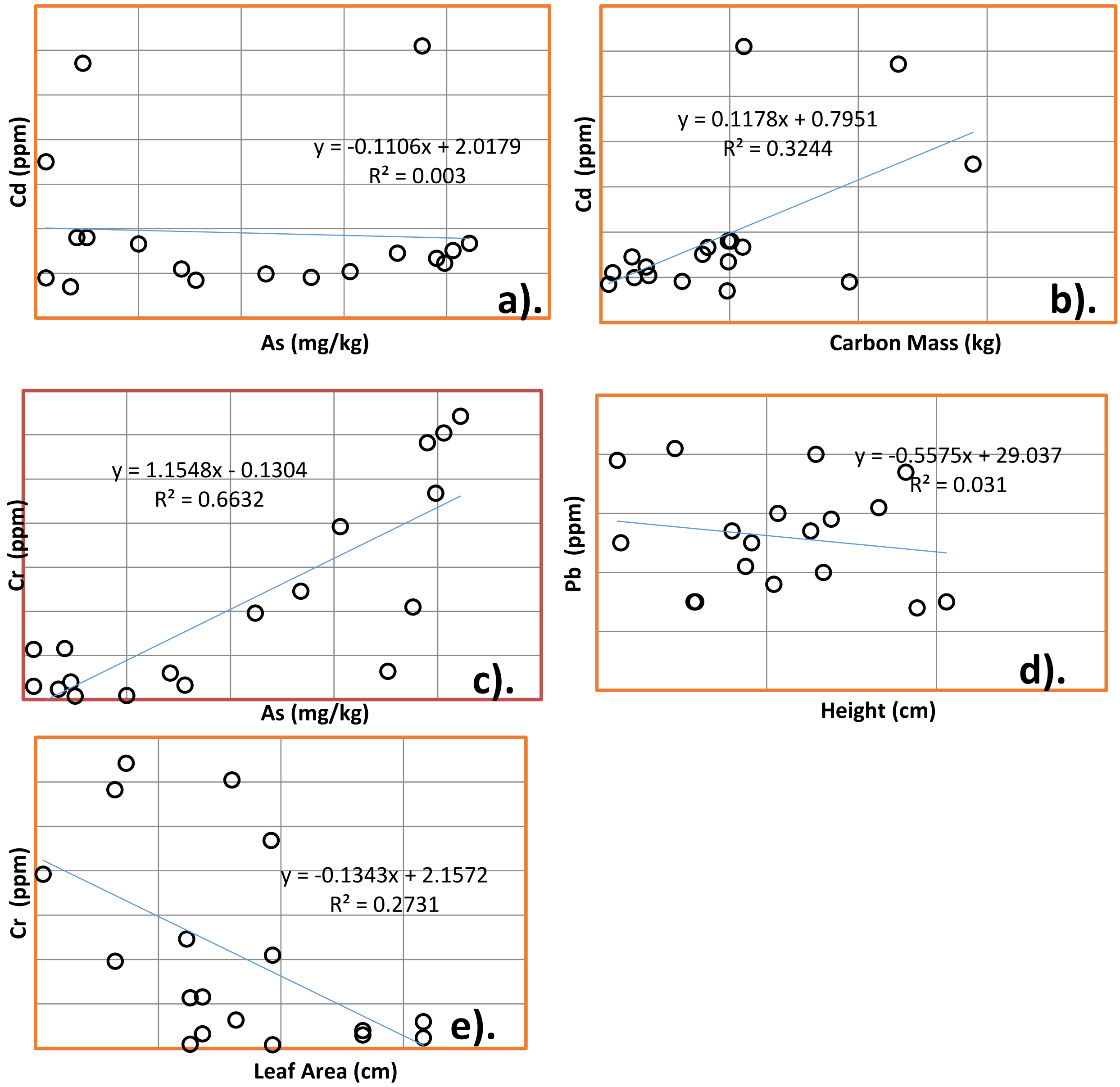

Figure 3. The scatter plot graph to explain the relationship between different parameters and also the correlation between them. Plot (a) shows the relationship between As and Cd, (b) between carbon mass and Cd, (c) between Cr and As, (d) between Pb and height, and (e) between Cr and leaf area.

Spatial distribution of different parameters

Spatial distribution patterns of heavy metal levels are shown as maps in **Figure 4**. In spatial distribution, data was presented in the form of maps to elaborate the trend and symmetry among different parameters that were studied or analyzed during the research discourse. It also represents the location of different samples on the map with their IDs and values of specific parameters. **Figure 4c** specifies the Pb levels of the tested species, as we discussed earlier Pb contamination showed a mix and match trend of increase or decrease, but is affected by the traffic exhaust.

Heavy metal contents of arsenic (As), chromium (Cr), and cadmium (Cd) are given spatial distribution pattern. **Figure 4a** also gives an un-even symmetry because of the random selection of tree species. Arsenic is mostly abundant in non-native species due to which As concentrations gave an irregular trend on the map. **Figure 4b** shows the distribution pattern of Cr that gave a specific pattern lower at the northern side, then higher at the southern side. The spatial distribution of Cd is given in **Figure 4c** and the map shows that Cd in northern side is higher than the Cd concentrations on the southern side, and Cd contents were found more abundant in the native species than that in the non-native ones.

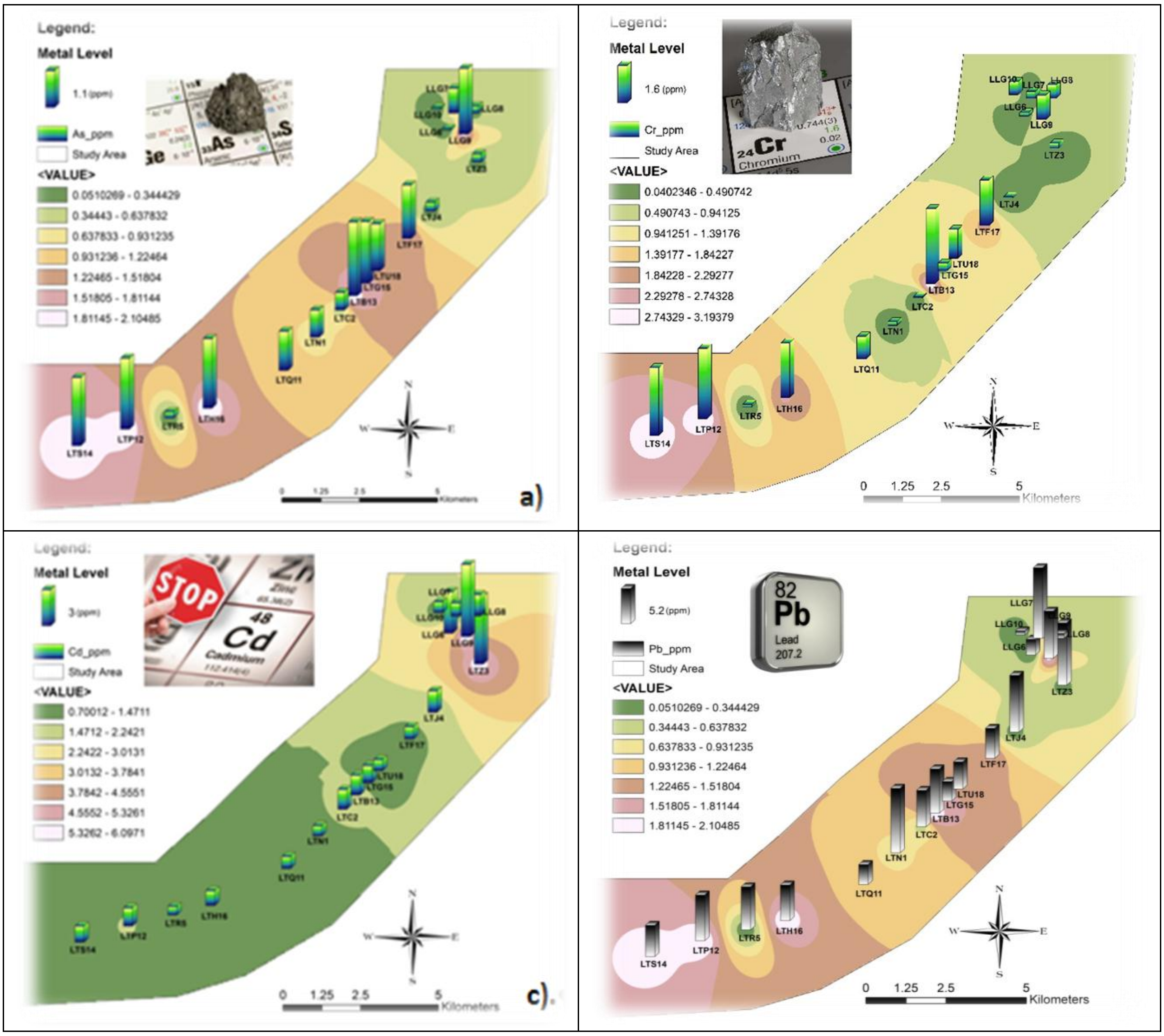

Figure 4. The map shows spatial allocation of heavy metal levels of (a) Arsenic, (b) Chromium, (c) Cadmium, (d) Lead based on analytical data using metal detecting instruments

Prominent concentrations of specific metals

From this study it was established that Pb and Cd concentrations in the samples were comparatively high in all leaf samples compared to As and Cr. The Pb content was almost higher in all leaf samples including the control samples (**Table 3**). Concentrations of both heavy metals in samples ranged from 10.3 to 0.6 mg/kg for Pb and from 6.1 to 0.7 mg/kg for Cd. This trend indicates that Pb is almost high at every site due to the massive flow of vehicular traffic in Lahore.

Physical attributes and metal concentrations trends

The Principal Component Analysis (PCA) plot (**Figure 5**) represents the results for the first and second components as they are statistically closely related. Lines direction and their closeness to each other show that heavy metal concentrations are dependent on different factors including concentrations of other metals, tree height, carbon mass, canopy, and leaf area. With PCA analysis direct or indirect relations between different parameters could be drawn in order to establish our opinion regarding which factors affect the metal levels in the leaves of different trees.

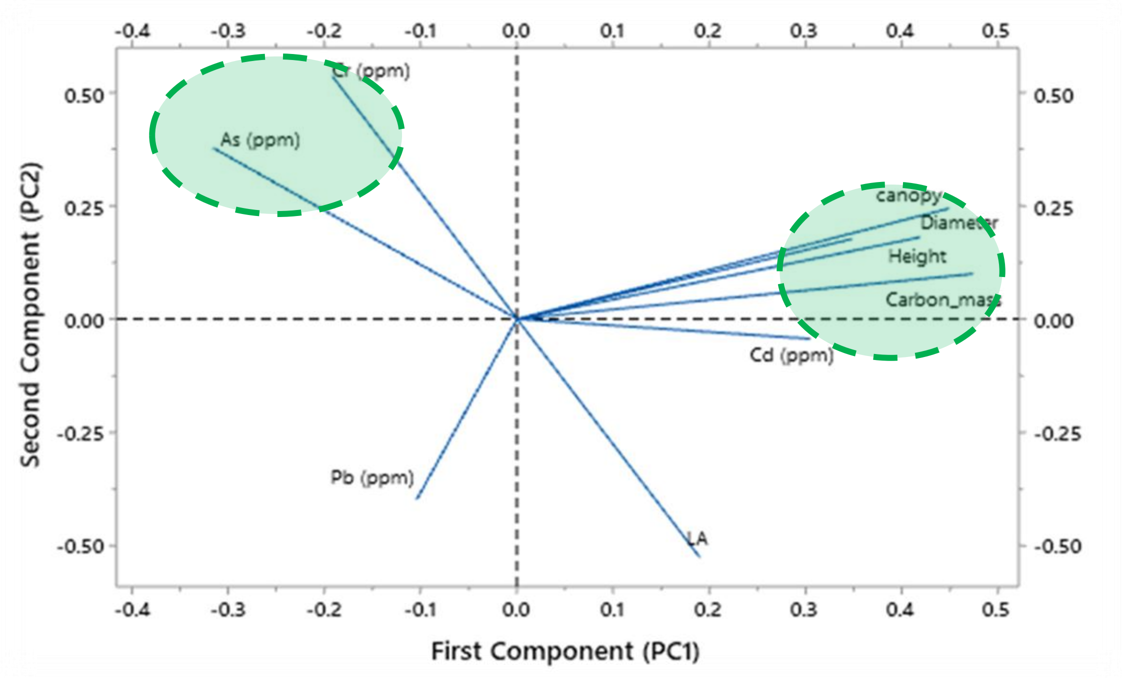

Figure 5. PCA shows a comparison of different parameters and their trends of clustering

Conclusion

In this investigation, the elemental concentrations of selected metals were found to be very variable, with no systematic change or spatial distribution pattern detected in the sampling area. Arsenic pollution (Average ~1.03 mg/kg) was found to be moderately lower, indicating less metal intake by the trees. Lead (Pb) accumulation levels in the leaves were found to be much higher in almost all samples (Average ~ 5.34 mg/kg) than the WHO recommended limit (2 mg/kg), with *Ficus religiosa* having the highest value among all samples. Chromium (Cr) contamination trends were documented to be high (Average ~ 1.06 mg/kg) in non-native species. Cadmium concentrations in the leaves were also found to be high (Average ~ 1.90 mg/kg) when compared to the WHO permitted limit (0.02 mg/kg) in plants. We infer from this study that the size of automobile traffic had a significant impact on heavy metal levels in the tree leaves as well as atmospheric compositions.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceived the idea: ZA, QZ, KS. Sampling from the field: KS. Writing of first draft: KS, QZ. Statistical analysis of data and drawing of figures: ZA, QZ, KS. Revision of the manuscript: ZA, KS, QZ.

Supplementary material
No supplementary material is included with this manuscript.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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