Pak. j. sci. ind. res. Ser. A: phys. sci. 2023 **66A**(1) 33-42

Assessment of Potential of Some Native Trees Along Busy Roads to Reduce the Air Pollution

Farhan Ahmad Khan Suri, Aisha Saleem Khan* and Saba Butt
Department of Life Sciences, Forman Christian College (A Chartered University), Zahoor Elahi Road, Gulberg III, Ferozepur Road, Lahore- 54600, Pakistan

(received November 29, 2021; revised June 29, 2022; accepted August 26, 2022)

Abstract. Vehicular emissions that contain traces of a variety of heavy metals are known to cause serious contamination of roadside plants and pose a risk to human health. In current research, some local trees growing along Canal road, Lahore, *Albizia procera* (Roxb. *Benth*., *Alstonia scholaris* (L.) *R.Br., Bougainvillea spectabilis* Willd., *Cassia fistula* L., *Ficus benghalensis* L. and *Ficus religiosa* L. were studied (morphology, chlorophyll estimation) to explore their potential in the phyto-remediation of harmful environmental pollutants like cadmium (Cd) and lead (Pb), through foliar route. Results showed degradation of chlorophyll content of all species except *B. spectabilis*, while reduction in cell sizes of only *A. procera, C. fistula* and *F. benghalensis*. Atomic absorption spectroscopy showed Pb accumulation in *A. procera, A. scholaris* and *C. fistula* and Cd accumulation in *B. spectabilis* only. Results suggest that these species can be used to reduce the level of Pb and Cd on busy road areas and therefore, improve quality of air.

Keywords: accumulation, cadmium, environment, foliar, lead, phyto-remediation.

Introduction

Environmental pollution is one of the biggest threats to developing countries around the world. Pakistan also faces the problem of environmental pollution which is due to toxic heavy metals. These are dense, naturally occurring elements that number almost 40 and have high atomic weights with different biological functions and chemical properties (Sharma and Agarwal, 2005). The agency for toxic substances and disease registry states in their guidelines that four heavy metals that include Cd and Pb are classified as being the most toxic due to their frequent occurance in the environment, toxicity and potential exposure to humans (Jamla *et al*., 2021).

Tons of heavy metals are annually released into the atmosphere and cause harm to the environmental ecology, ecosystem and to human health. They are also absorbed into the soil, causing a pH change and alter plants' physiological, anatomical and reproductive attributes (Altaf *et al*., 2021).

Vehicular exhaust emission has been considered one of the most prevalent causes of these heavy metals (Sarhan *et al*., 2021) and inflict harmful effects on roadside vegetation (Altaf *et al*., 2021). Some secondary anthropogenic sources include mining processes, human activities, traffic, petrochemical by products combustion, industrial sources diffuse and smelter sources (Pujari and Kapoor, 2021). In Pakistan as well, particularly Lahore, there has been a massive rise in air pollution that contains toxic heavy metals like aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), silicon (Si) and zinc (Zn) spread into the atmosphere (Schneidemesser *et al*., 2010). A recent study also finds that the concentration of some elements and toxic metals that include Cd, Cu, Cr and Pb in the city of Lahore are 6 % - 9 % higher than the recommended WHO guidelines (Khanum *et al*., 2020). Additionally, Lahore is ranked the most polluted city in Pakistan according to the IQAir's (2021) in the air quality index (AQI), which has led to problems like asthma and toxicity in people (Raza *et al*., 2021). Chronic deposition of even lower rates of heavy metals lead to accumulation in environments which pose hazards to human health and the environment (Fang *et al*., 2021).

Heavy metals accumulate within plants and hinder their metabolic processes (Mahdu and Sadagopan, 2020). A study by Uka *et al*. (2021)also confirms that level of photosynthetic pigments of trees along roadsides, where there is vehicular exhaust, decreases due to contamination of air. On exposure of high concentrations of these heavy metals, growth patterns of plants and different stages of development are negatively affected.

*Author for correspondence;
E-mail: aishasaleemkhan@fccollege.edu.pk

Heavy metals target a multitude of plant mechanisms, organelles like chloroplasts, photosynthetic enzymes, movement of stomata and the rate of transpiration. They may also be accumulated in different parts of the plant, there with reducing biomass and protein production. Pb specifically, when enters the plant may alter the permeability of membrane, regulation of hormones and cause stunted growth (Jamla *et al*., 2021). It also affects the metabolic activities of plants and disrupt their developmental stages by hampering with their biochemical pathways (Khan *et al.*, 2016). These non-essential heavy metals (Pb and Cd) affect plants' essential element absorption and transport, disrupt metabolism and impact their development (Cheng, 2003). Heavy metal toxicity is also dependent on several factors that include quantity, exposure route, genetics etc. as well as their reactivity potential and capacity of oxidation (Jamla *et al*., 2021) and they pose a high degree of it to health of humans and the environment (Tchounwou *et al*., 2012). Additionally, toxic levels of heavy metals in plants are capable in interacting with biomolecules like DNA and nuclear proteins which leads to the increase of excessive reactive oxygen species (ROS). This results in physiological, morphological and anatomical anomalies in plants like chlorosis, protein degradation and lipid peroxidation (Emamverdian *et al.*, 2015).

It has become mandatory to use remediation in controlling pollution containing heavy metals for the sake of human and environmental health. Many strategies have been developed to tackle this issue. One strategy to reduce the environmental pollution is phyto-remediation (Wei *et al.,* 2021). The ecofriendly process of bio-remediation that uses diverse types of plants to stabilize, transfer, reduce, remove and/or destroy the contaminants present around them (Yaashikaa *et al*., 2022; Ali *et al*., 2013) which has become a developmental hotspot for heavy metal phyto-remediation (Yang *et al*., 2022). Plants have been known to use their own metabolism and other interactions with micro-organisms to remediate the environment they persist in (Wei *et al.,* 2021). Although the bio-accumulation of heavy metals by plants may affect their growth, area of leaf, formation of chlorophyll and disrupt their contents of sugar and vitamins, different plants have developed various mechanisms to deal with that bio-accumulation. In addition, many conditions also affect the ability of plants to purify their environment e.g., species of plant, intensity of light, temperature, conductance of stomata and microbial species (Wei *et al*., 2021*)*. Some may even be categorized as hyper accumulators, that have the ability to absorb heavy metals in excess amounts and discharge them from the soil they are planted in (Guo *et al*., 2020).They include processes like phyto-degradation in which plants metabolize and destroy the contaminants in their tissues and phyto-accumulation (also known as phyto-extraction) in which contaminants are absorbed from the roots of the plant simultaneously with water and other nutrients, either in stable forms or by converting them into non-toxic forms. Mass of the contaminant is not destroyed but is trans-located to the plant's shoots and leaves and this method is used primarily for heavy metal uptake (Shen *et al*., 2022; Lee, 2013). An eco-sustainable filter i.e., tree leaves can be used to remove heavy metals in massive quantities and other particulate matter in the air to improve quality of air in highly polluted areas (Fang *et al*., 2021). Though, it is also known that plants can transport and accumulate heavy metals from the soil *via* the roots to the leaves as well (Yaashikaa *et al*., 2022). Since the method of phyto-remediation is cost effective, ecofriendly and more efficient, it is a suitable solution to the problems because it will remove or reduce the concentration amount of heavy metal in the atmosphere. Furthermore, heavy metals like As, Cd and Pb have already been reported to enter plant leaves *via* foliar transfer (Wang *et al*., 2018) and some plant species are already known to tolerate and remediate high levels of heavy metal pollution i.e., roadside plant, which highlight their capability of being mass planted (Altaf *et al*., 2021).Study shows that the urbanization rate in Pakistan is approximately 38.6%. Hence, roads with a higher frequency of traffic emit heavy metal pollution in higher levels, with its lack of awareness and limited resources, Pakistan is desperately trying to regulate and decrease the pollution made by vehicles (Shirwani *et al*., 2020).

Some research has been done on the selected tree species already. In one research, *B. spectabilis* was seen to store Cd and was also known to produce citric acid that detoxifies Cd and control its concentration (Wang *et al.,* 2018). An approach that integrated biophysical based and physiological indicators by Singh (2021) determined *A. scholaris* to be a well tolerant species to the emissions from vehicles and recommended as a roadside plantation to mitigate air pollution.

Additionally, *A. scholaris*; *B. spectabilis*; *C. fistula*; *F. benghalensis* and *F. religiosa* have been reported to be well thriving species, tolerant to industrial air pollution, auto mobile emissions and could be recommended as roadside plants that could also absorb or amass heavy metals from the air and control particle matter, while still staying healthy (Rai, 2016; Gupta *et al*., 2011; Lakshmi *et al*., 2009; Pal *et al*., 2002).

The purpose of this research was to assess some different types of local plant species, namely *A. procera; A. scholaris; B. spectabilis; C. fistula; F. benghalensis* and *F. religiosa* for their heavy metal absorption and accumulation potential through the foliar route (leaves) and if possible, to identify suitable air phyto-remediators for cleaning the heavy metal polluted environment. It could also help us to identify which of the local tree species chosen are potential 'hyper-accumulators' of heavy metals and could further aid in phyto-remediation. Furthermore, if potential phyto-remediator tree species are identified, they can be recommended to be grown in the busy road or pollution ridden areas of Lahore to lessen the levels of air pollution and improve the general environment.

Materials and Methods

Hand sectioning was used to prepare anatomical sections of leaf samples, the upper and lower epidermis cell sizes and sizes of guard cells were measured for the experimental groups and then compared to the control group. Content of chlorophyll for the experimental groups and control group was estimated through spectrophotometry. To quantify the accumulated heavy metals in all the groups atomic absorption spectroscopy was done and the results were compared between the experimental and control group. To achieve the objectives of this research, the following methodologies were used:

Sample collection. Samples of leaves were collected of following species from different locations in Lahore; *A. procera* (white siris); *A. scholaris* (blackboard tree); *B. spectabilis* (paper flower); *C. fistula* (golden shower/amaltas); *F. benghalensis* (banyan fig) and *F. religiosa* (peepal tree). Two major groups were compared: plants that grew in the areas of high levels of environmental air pollution i.e different locations of busy roads on Canal road, Lahore and plants that grew in the area of low-to-negligible level of environmental air or soil pollution, far from vehicular exhaust at residential areas and FCCU Botanical Garden, Lahore. Leaf sampling was done during day time of vehicular movement on road. For the control group, samples of leaves of corresponding species were collected during same time of day. In order to observe and analyze how all the selected species respond to environmental pollution (potentially containing toxic heavy metals) conditions were kept similar.

Morphology. The external morphology parameters i.e leaf shape, colour and texture were observed and compared for all the samples. The epidermis of leaves was studied to observe differences in stomata using a light microscope. Slides of transverse and longitudinal sections of leaves were prepared, observed and photographed under light microscope (MT5300H – Meiji Techno, Japan). All the observations were recorded at 10X and 40X power. An ofular micrometer was used to measure the length and width of cells of upper and lower epidermis and guard cells. The data was recorded and with the aid of a stage micrometer it was converted into micron meters (μm).

Stomatal counts. Lower epidermis of leaves were observed with the direct method approach of counting stomata under microscope. Small, peeled sections were taken from the leaf epidermis, stained in toluidine blue for a few minutes and then studied under the light microscope at 40X magnification and the number of open stomatal pores were recorded.

Internal morphology. Transverse, abaxial and adaxial epidermis section slides of leaves were prepared. They were cut by hand sectioning and stained by toluidine blue and viewed under a light microscope. The length and width of upper and lower epidermal cells and stomatal guard cells were recorded.

Chlorophyll estimation. Acetone was used as the solvent. A 100 mg leaf tissue sample was cut with a blade into small pieces. Using a pastel and mortar containing 4 mL of 80% acetone, it was then crushed for 3-5 min until devoid of green colour. It was further rinsed with 2 mL acetone and the final concentration was raised to 10 mL using acetone (80 %). Then, transferred to a centrifuge tube and centrifuged also at 10,000 rpm for 5 min. After centrifugation, 3 mL of the supernatant was extracted and transferred to a cuvette which was then analyzed for chlorophyll 'a' and 'b' present at different wavelengths by a UV/VIS spectrophotometer. Chlorophyll estimation was done by the Arnon's (1949) method to determine the concentration of chlorophyll.

In spectrophotometry, acetone was used as a blank. For estimating chlorophyll 'a' and 'b' the absorbance of samples at wavelengths of 663 nm and 645 nm respectively were recorded.

Chlorophyll ‘a’ (mg/g) = [(12.7 × A663) - (2.69 × A645)] × mL of acetone / mL of leaf tissue

Chlorophyll ‘b’ (mg/g) = [(22.9 × A645) - (4.68 × A663)] × mL of acetone / mL of leaf tissue.

Total chlorophyll (mg/g) = Chl ‘a’ + Chl ‘b’ (Arnon, 1949).

Atomic absorption spectroscopy (AAS). To scientifically compare the amounts of heavy metals present in experimental and control samples atomic absorption spectroscopy was done for quantification. 0.1 g of leaf samples of species were cut and weighed to be individually heated in a 25 mL concentrated nitric acid and 10 mL concentrated sulphuric acid mixture (Hseu, 2004). All samples in beakers were carefully heated under fume hood. The sample mixture was stirred at intervals for a few min until discoloration was seen and no visible suspension was left. The flame was then turned off and 15 mL of distilled water was added to the beaker and solution made up to 50 mL. After some time was given to the mixture to cool, 50 mL of distilled water was added to make the solution up to 100 mL. It was then transferred to a volumetric flask. Then, 15-20 ml of this solution was filtered and tested for heavy metal analysis. The same procedure was followed for all the samples for AAS.

Results and Discussion

A. procera. In the current work, the leaves of *A. procera* collected from busy road areas showed an insignificant decrease in chlorophyll content (Table 1) and a slightly greater number of stomata which indicates a resistance of plant to heavy metals in response to air pollution (Table 2). Sizes of cells of leaves from busy roads showed a decrease as compared to the control (Table 2, Fig. 1). Another study reported similar results (Kapoor *et al*., 2019), where the effects of Cd treatment caused decrease in morphological characteristics of *A. procera*. In this work *A. procera* leaves accumulated a relatively high amount of Pb, 22.65µg/Kg (Fig. 2). Therefore, it can potentially be used to reduce the level of Pb in the air as a Pb bio-indicator species.

A. scholaris. Results showed a significant decrease in the amount of chlorophyll content in leaves of *A. scholaris* from busy roads as compared to control (Table 1), which is also reported in a study by Singh (2021). This might be due to accumulation of heavy metals within leaf tissues. A study that further supports the result states that the heavy metals cause disruptions

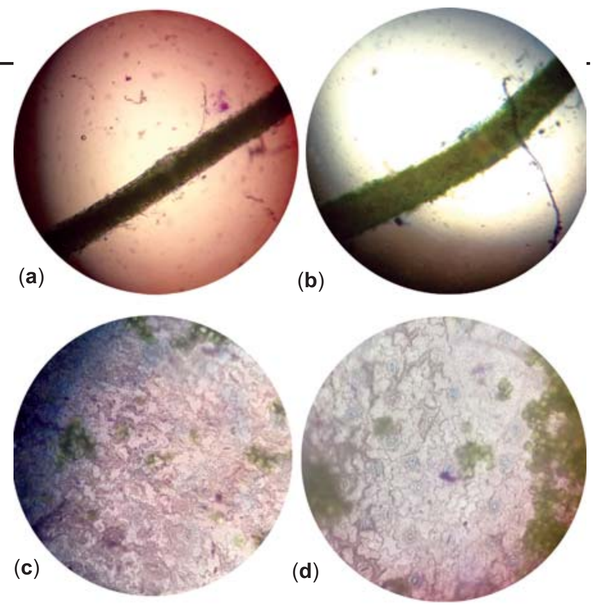

Fig. 1. Cross section (10X) and lower epidermis of leaf (40X) of *A. procera*:(**a**) and (**c**) Control, (**b**) and (**d**) from busy road area.

Table 1. Chlorophyll estimation for species from control area and busy road areas

Species	*A. procera*		*A. scholaris*		*B. spectabilis*		*C. fistula*	
	Chl ‘a’	Chl ‘b’	Chl ‘a’	Chl ‘b’	Chl ‘a’	Chl ‘b’	Chl ‘a’	Chl ‘b’
Control	18.63 ± 1.2	33.88 ± 3.4	20.60 ± 1.9	32.71 ± 2.7	7.63 ± 0.75	13.86 ± 0.98	20.32 ± 2.0	35.80 ± 2.4
Busy road #1	15.34 ± 0.9	30.43 ± 2.6	18.24 ± 1.9	29.11 ± 2.3	12.32 ± 0.87	21.09 ± 1.2	18.23 ± 1.9	27.96 ± 2.4
Busy road #2	16.23 ± 2.3	28.22 ± 2.9	6.33 ± 0.8	12.34 ± 2.1	12.34 ± 0.86	19.38 ± 1.2	12.34 ± 0.83	15.56 ± 1.5
Busy road #3	13.92 ± 0.9	25.29 ± 1.1	5.30 ± 0.9	9.63 ± 0.7	11.90 ± 0.82	21.62 ± 1.3	11.42 ± 0.54	11.37 ± 0.9

Chlorophyll estimation (mg/g)

Table 2. Stomatal counts for *A. procera* from control area and busy road areas

Location	Upper epidermal cells (µm)		Lower epidermal cells (µm)		Guard cell (µm)		Stomatal count
	L	W	L	W	L	W	
Control	15.3 ± 0.78	12.2 ± 0.66	17.2 ± 0.87	12.5 ± 0.74	9.2 ± 0.56	2.8 ± 0.65	32 ± 0.8
Busy road #1	14.2 ± 0.50	10.2 ± 0.23	14.8 ± 0.75	11.8 ± 0.43	9.0 ± 0.44	2.8 ± 0.76	34 ± 1.5
Busy road #2	15.1 ± 1.24	11.7 ± 0.67	14.2 ± 0.66	8.3 ± 0.77	9.0 ± 0.65	2.3 ± 0.43	33 ± 1.2
Busy road #3	13.7 ± 0.89	11.4 ± 0.87	12.9 ± 0.76	7.1 ± 0.55	8.6 ± 0.67	2.5 ± 0.61	34 ± 0.9

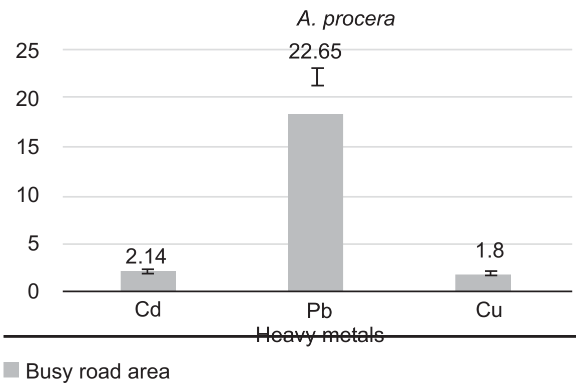

Fig. 2. Amount (µg/Kg) of accumulated heavy-metals in leaf of *A. procera* through AAS.

in the biological systems in cells due to Mg^{2+} substitutions and induces high chlorophyllase activity (Gupta *et al*., 2011). There was an evident decrease in the observed stomatal counts of leaves from busy road areas as compared to control (Table 3), which indicates a decrease in metabolic activity and inhibition of stomatal pores. The sizes of cells in adaxial and abaxial surfaces from leaves of *A. scholaris* frombusy road area showed a decrease in width but an increase in length (Table 3, Fig. 4). The irregular changes in size could be the effect of heavy metals accumulated differently by leaf surfaces. *A. scholaris* was reported to have high tolerance to heavy metals and high metal accumulation factors of Cd and Pb and was recommended as being one of the "best variety" to be planted along road sides in heavily polluted areas (Singh, 2021; Tak and Kakde, 2019; Gupta *et al*., 2011). In the current work *A. scholaris* from busy road areas was reported to accumulate a relatively high amount of Pb in leaves, 18.32 µg/Kg (Fig. 3) and can potentially be used as an accumulator to reduce the levels of Pb in the air, or function as a Pb bio-monitor species.

***B. spectabilis*.** In the current work, surprisingly, the *B. spectabilis* leaves collected from the busy road areas showed a note worthy increase in content of chlorophyll as compared to control (Table 1). It suggests the *B. spectabilis* was more metabolically active and tolerant to polluted air. One study that supports this claim was done (Roy *et al*., 2020) in which they mention that species with higher levels of chlorophyll are more tolerant to an environment with contaminants and are favourable to plant in that area. The cell sizes of the upper epidermis and guard cells of leaves from busy

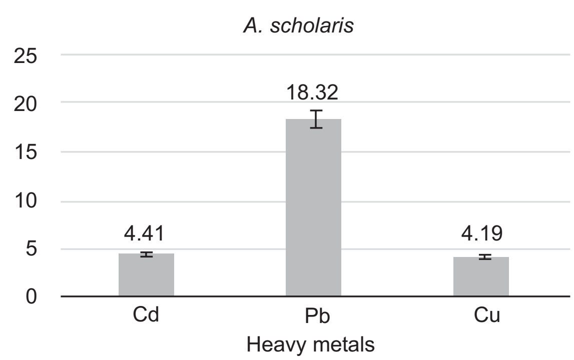

Fig. 3. Amount (µg/Kg) of accumulated heavy metals in leaf of *A. scholaris* through AAS.

Table 3. Stomatal counts for *A. procera* from control area and busy road areas

Location	Upper epidermal cells (µm)		Lower epidermal cells (µm)		Guard cell (µm)		Stomatal count
	L	W	L	W	L	W	
Control	21.87 ± 1.04	26.25 ± 1.10	12.65 ± 0.85	27.6 ± 1.12	15.76 ± 0.95	21.87 ± 1.04	55 ± 1.4
Busy road #1	22.45 ± 2.10	23.5 ± 1.22	12.98 ± 0.55	25.3 ± 2.02	17.85 ± 1.09	22.45 ± 2.10	37 ± 0.9
Busy road #2	25.59 ± 1.23	20.42 ± 1.05	16.23 ± 1.30	22.9 ± 1.20	15.86 ± 0.98	25.59 ± 1.23	39 ± 2.3
Busy road #3	26.25 ± 1.09	17.4 ± 0.99	18.76 ± 1.02	22.5 ± 1.08	18.23 ± 1.01	26.25 ± 1.09	36 ± 0.8

roads areas showed an increase, whereas the lower epidermis showed a decrease as compared to controls (Table 4, Fig. 5). Stomatal counts showed a slight decrease in samples from busy road areas (Table 4). *B. spectabilis* has been reported to be a well thriving species in peri-urban areas, is a tolerant species to mobile emissions even with significant leaf morphological changes and has potential in accumulating heavy metals and controlling air pollution in heavily polluted urban sites (Rai, 2016; Pal *et al*., 2002). Its leaves have been observed to accumulate Cd and Pb (Juson *et al*., 2016) and store and detoxify Cd by citric acid to control its concentration (Wang *et al*., 2018). Based on current work, atomic absorption spectrometry results showed that leaves of *B. spectabilis* accumulated a relatively high amount of Cd, 42.24 µg/Kg (Fig. 6), suggesting

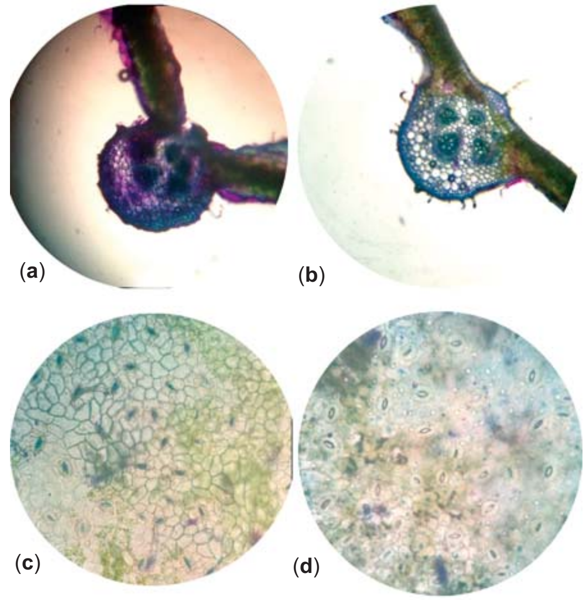

Fig. 5. Cross section (10X) and lower epidermis of leaf (40X) of *B. spectabilis*:(**a**) and (**c**) Control, (**b**) and (**d**) from busy road area.

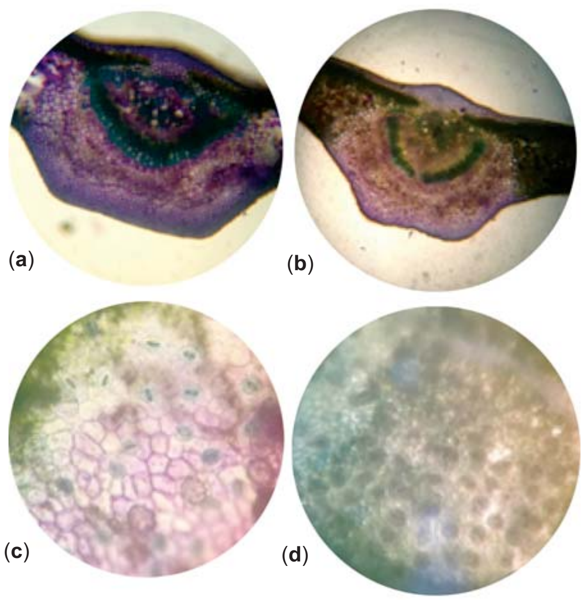

Fig. 4. Cross section (10X) and lower epidermis of leaf (40X) of *A. scholaris*: (**a**) and (**c**) Control, (**b**) and (**d**) from busy road area.

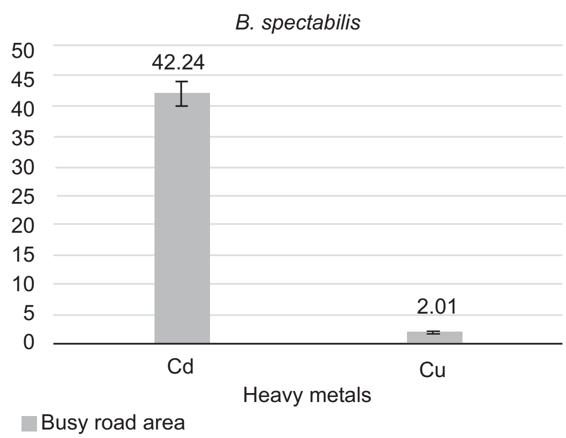

Fig. 6. Amount (µg/Kg) of accumulated heavy metals in leaf of *B. spectabilis* through AAS.

Table 4. Stomatal counts for *B. spectabilis* from control area and busy road areas

Location	Upper epidermal cells (µm)		Lower epidermal cells (µm)		Guard cell (µm)		Stomatal count
	L	W	L	W	L	W	
Control	27.5 ± 1.13	21.86 ± 1.07	31.25 ± 1.12	21.87 ± 1.08	16.75 ± 1.00	6.87 ± 0.91	35 ± 1.2
Busy road #1	32.5 ± 2.20	23.2 ± 1.87	26.87 ± 1.04	19.23 ± 1.23	19.02 ± 0.99	8.03 ± 0.87	30 ± 0.7
Busy road #2	29.3 ± 1.81	23.9 ± 1.32	30.02 ± 1.43	14.76 ± 1.73	17.97 ± 1.07	7.04 ± 1.01	32 ± 1.0
Busy road #3	31.21 ± 1.12	24.36 ± 1.07	25.34 ± 1.08	13.21 ± 1.03	19.76 ± 0.98	8.43 ± 0.99	29 ± 0.8

that the plant can potentially be used to store and reduce the levels of Cd in air pollution on busy road areas.

C. fistula. In the current work, the leaves of *C. fistula* from busy road area showed significant decrease in stomatal counts (Table 5) and chlorophyll content (Table 1) that suggest stomata and chlorophyll were inhibited. This could possibly be because of the degradation of structure of chlorophyll due to a higher chlorophyllase activity or Mg^{2+} ions being substituted because of high environmental air pollution (Gupta *et al*., 2011). A decrease in overall cell sizes was observed in the leaves collected from busy road areas (Fig. 8). This could be the sign of heavy metal toxicity as *C. fistula* in the current work has been observed to accumulate a relatively high amount of Pb in its leaf tissue, 27.3 µg/Kg (Fig. 7). Hence, *C. fistula* can potentially be used to reduce the levels of Pb in the atmosphere. In other studies, it has been found to be significantly tolerant to industrial air pollution (Lakshmi *et al*., 2009), and automobile exhausts reported by (Pal *et al*., 2002) which could be recommended as a roadside plant and absorb heavy metals, while staying healthy. Its yellow hanging flowers could also decorate the roads in the form of an ornamental plant.

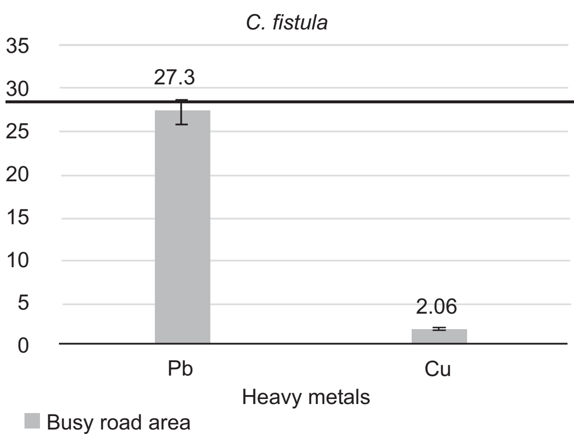

Fig. 7. Amount (µg/Kg) of accumulated heavy metals in leaf of *C. fistula* through AAS.

F. benghalensis. *F. benghalensis* has been reported to be a well-thriving species, accumulate high levels of Pb in its leaves, is intermediately tolerant to pollution in air and has one of the best dusts capturing capacities. So, can be used as a potential control for heavy metal pollution around heavily polluted urban sites and road sides (Roy *et al*., 2020; Rai, 2016; Datta and Ghosh, 1985) The cell sizes and stomatal counts in leaves from busy road areas showed a very notable decrease as compared to control (Table 6). This might be an indication of heavy metal toxicity from air pollution.

F. religiosa. *F. religiosa* has been observed to be very tolerant to auto-exhaust and air pollution even with significant leaf morphological changes, had one of the best dusts capturing capacities, and was known to be a good hyper accumulator of Cd and Cu (Roy *et al*., 2020; Patel *et al*., 2015; Pal *et al*., 2002). Significant morphological changes were observed in the current study. Contrary to previous studies (Yang *et al*., 2004), a noteworthy increase in stomatal counts was also detected in leaves from busy roads areas as compared

Table 5. Stomatal counts for *C. fistula* from control area and busy road areas

Location	Upper epidermal cells (µm)		Lower epidermal cells (µm)		Guard cell (µm)		Stomatal count
	L	W	L	W	L	W	
Control	25.41 ± 1.12	12.52 ± 0.89	12.75 ± 0.87	27.5 ± 1.05	4.68 ± 0.67	16.98 ± 0.88	124 ± 8.3
Busy road #1	18.90 ± 2.7	11.27 ± 0.92	10.17 ± 0.98	15.65 ± 2.41	4.22 ± 0.33	14.09 ± 0.97	75 ± 7.2
Busy road #2	13.23 ± 0.89	13.22 ± 1.23	10.83 ± 0.87	11.22 ± 0.93	3.23 ± 0.87	10.32 ± 0.65	87 ± 6.9
Busy road #3	12.99 ± 0.97	16.25 ± 1.10	9.46 ± 0.78	9.25 ± 0.82	3.48 ± 0.76	10.1 ± 0.98	75 ± 9.3

Table 6. Stomatal counts for *F. benghalensis* from control area and busy road areas

Location	Upper epidermal cells (µm)		Lower epidermal cells (µm)		Guard cell (µm)		Stomatal count
	L	W	L	W	L	W	
Control	84.37 ± 8.87	25.3 ± 1.12	128.87 ± 16.23	97.7 ± 12.43	20.31 ± 1.23	9.37 ± 0.97	139 ± 6.4
Busy road #1	61.24 ± 5.31	11.34 ± 1.04	22.97 ± 7.22	20.38 ± 4.30	10.66 ± 1.06	8.45 ± 0.87	108 ± 7.3
Busy road #2	30.38 ± 4.62	17.77 ± 2.31	42.65 ± 9.32	23.54 ± 3.55	12.87 ± 0.98	6.22 ± 0.50	99 ± 5.5
Busy road #3	21.87 ± 1.11	9.37 ± 0.98	18.77 ± 1.07	15.50 ± 1.08	8.43 ± 0.87	6.25 ± 0.69	105 ± 6.7

Table 7. Stomatal counts for *F. religiosa* from control area and busy road areas

Location	Upper epidermal cells (μm)		Lower epidermal cells (μm)		Guard cell (μm)		Stomatal count
	L	W	L	W	L	W	
Control	40.0 ± 2.23	31.25 ± 1.89	25.4 ± 1.20	15.3 ± 0.98	29.68 ± 1.98	7.81 ± 0.97	34 ± 3.4
Busy road #1	47.3 ± 3.22	42.7 ± 1.99	29.4 ± 1.09	22.5 ± 1.02	30.28 ± 1.87	7.98 ± 1.2	41 ± 3.2
Busy road #2	52.7 ± 3.65	55.33 ± 2.54	38.7 ± 2.03	29.8 ± 0.99	33.87 ± 2.3	10.03 ± 0.86	38 ± 2.3
Busy road #3	50.2 ± 3.00	56.25 ± 3.56	40.62 ± 2.33	31.25 ± 1.89	34.3 ± 1.78	9.37 ± 0.99	46 ± 4.5

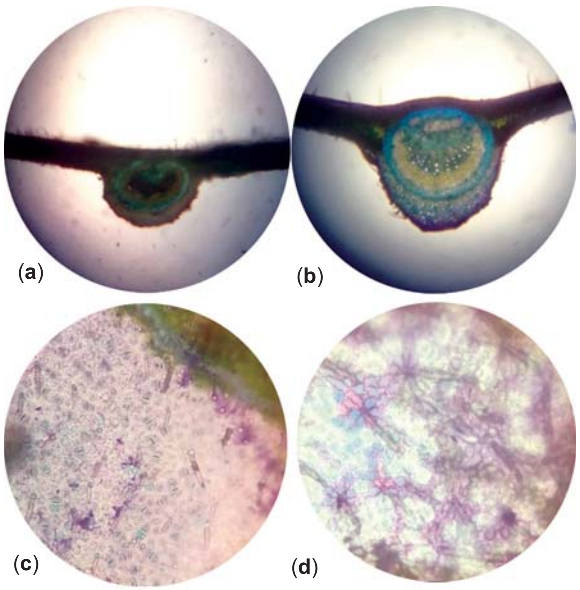

Fig. 8. Cross section (10X) and lower epidermis of leaf (40X) of *C. fistula*:(**a**) and (**c**) Control, (**b**) and (**d**) from busy road area.

to control (Table 7). The sizes of cells in leaves from busy road areas also showed a significant increase as compared to control (Table 7) which indicates tolerance and resistance of the plant to air pollution and heavy metal stress (Roy *et al*., 2020).

In controls, none of the species showed any traces of the studied heavy metals.

Conclusion

Accumulation of Pb in *A. procera*, *A. scholaris* and *C. fistula* suggests that these species could be used as potential accumulators to remove Pb from the air. This idea is supported by the anatomical parameters as reduction in cell sizes was observed but not significantly. Chlorophyll estimation and atomic absorption spectroscopy further supported that Pb can be accumulated in their leaves. Accumulation of Cd in *B. spectabilis* without showing visible symptoms of Cd toxicity suggests that it could be used as a potential accumulator to remove Cd from the air. Anatomical parameters support that the species had increased cell sizes, hence less affected by the heavy metals. Chlorophyll estimation showed increased chlorophyll and atomic absorption spectroscopy supports that Cd can be accumulated in *B. spectabilis* leaves. However, roles of these species can be further studied at a molecular level for their potential in phyto-remediation through foliar route. In addition, these species can also be used as ornamental plants; to decorate roadways, aside from their phyto-remediation and medicinal qualities.

Acknowledgment

The authors acknowledge the Department of Biological Sciences, Forman Christian College (A Chartered University) for the access to laboratories and equipment. We would also like to acknowledge the support and encouragement of Chairperson of the School of Life Sciences, FCCU, Dr. Samina Mehnaz and to the Dean of the School of Life Sciences, FCCU; Dr. Kausar Abdullah Malik for the opportunity to conduct this research work.

Conflict of Interest. The authors declare they have no conflict of interest.

References

Ali, H., Khan, E., Sajad, M.A. 2013. Phyto-remediation of heavy metals concepts and applications. *Chemosphere*, **91:** 869-881.

Altaf, R., Altaf, S., Hussain, M., Shah, R.U., Ullah, R., Ullah, M.I., Rauf, A., Ansari, M.A., Alharbi, S.A., Alfarraj, S., Datta, R. 2021. Heavy metal accumulation by roadside vegetation and implications for pollution control. *Plos one*, **16:** 1-15.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *PlantPhysiology*, **24:** 1-15.

Cheng, S. 2003. Effects of heavy metals on plants and resistance mechanisms. *Environmental Science and Pollution Research*, **10:** 256-264.

Datta, S.C., Ghosh, J.J. 1985. A study of the distribution pattern of lead in the leaves of banyan trees (*Ficus benghalensis*) from different traffic density regions of Calcutta. *Ecotoxicology and Environmental Safety*, **9:** 101-106.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y. 2015. Heavy metal stress and some mechanisms of plant defense response. *The Scientific World Journal*, **2015:** https://doi.orig110.1155/2015/756120.

Fang, T., Jiang, T., Yang, K., Li, J., Liang, Y., Zhao, X., Gao, N., Li, H., Lu, W., Cui, K. 2021. Bio-monitoring of heavy metal contamination with roadside trees from metropolitan area of Hefei, China. *Environmental Monitoring and Assessment*, **193:** 151. doi:10.1007/510661-021-0892-1

Guo, H., Jiang, J., Gao, J., Zhang, J., Zeng, L., Cai, M., Zhang, J., 2020. Evaluation of cadmium hyperaccumulation and tolerance potential of *Myriophyllum aquaticum*. *Ecotoxicology and Environmental Safety*, **195:** 11050.

Gupta, S., Nayek, S., Bhattacharya, P. 2011. Effect of air-borne heavy metals on the biochemical signature of tree species in an industrial region, with an emphasis on anticipated performance index. *Chemistry and Ecology*, **27:** 381-392.

Hseu, Z.Y. 2004. Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresource Technology*, **95:** 53-59.

IQAir *Air Quality in Pakistan*, 2021, 1-9. https://www.iqair.com/pakistan

Jamla, M., Khare, T., Joshi, S., Patil, S., Penna, S., Kumar, V. 2021. Omics approaches for understanding heavy metal responses and tolerance in plants. *Current Plant Biology*, **27:** 100213.

Juson, A.E., Martinez, M. K., Ching, J.A. 2016. Accumulation and distribution of heavy metals in *Leucaena leucocephala* Lam. and *Bougainvillea spectabilis* Willd. plant systems. *Journal of Experimental Biology and Agricultural Sciences*, **4:** 1-6.

Kapoor, D., Singh, M.P., Kaur, S., Bhardwaj, R.Z.B., Sharma, A. 2019. Modulation of the functional components of growth, photosynthesis, and anti-oxidant stress markers in cadmium exposed *brassica juncea* L. *Plants*, **8:** 260.

Khan, A.S., Hussain, M.W., Malik, K.A. 2016. A possibility of using waterlily (*Nymphaeaalba l.*) for reducing the toxic effects of chromium (Cr) in industrial wastewater. *Pakistan Journal of Botany*, **48:** 1447-1452.

Khanum, F., Chaudhry, M. N., Skouteris, G., Saroj, D., Kumar, P. 2020. Chemical composition and source characterization of PM10 in urban areas of Lahore, Pakistan. *Indoor and Built Environment*, **30:** 924-937.

Lakshmi, P.S., Sravanti, K.L., Srinivas, N. 2009. Air pollution tolerance index of various plant species growing in industrial areas. *Ecoscan*, **2:** 203-206.

Lee, J. H. 2013. An overview of phyto-remediation as a potentially promising technology for environmental pollution control. *Biotechnology and Bioprocess Engineering*, **18:** 431-439.

Madhu, P.M., Sadagopan, R.S. 2020. Effect of heavy metals on growth and development of cultivated plants with reference to cadmium, chromium and lead–a review. *Journal of Stress Physiology and Biochemistry,* **16:** 84-102.

Pal, A., Kulshreshtha, K., Ahmad, K.J., Behl, H.M. 2002. Do leaf surface characters play a role in plant resistance to auto-exhaust pollution? *Flora-Morphology, Distribution, Functional Ecology of Plants*, **197:** 47-55.

Patel, K.S., Sharma, R., Dahariya, N.S., Yadav, A., Blazhev, B., Matini, L., Hoinkis, J. 2015. Heavy metal contamination of tree leaves. *American Journal of Analytical Chemistry*, **6:** 687-693.

Pujari, M., Kapoor, D. 2021. Heavy metals in the environment: *Impact, Assessment and Remediation*, Edited Kumar, V., Sharma. A., Cerda, A. pp 1-37, Publisher Elsevier Inc., New York, USA.

Rai, P.K. 2016. Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. *Journal of Asia-Pacific Biodiversity*, **9:** 47-55.

Raza, H., Bibi, T., Bibi, H., Asim, M.J., Bilal, H., Rasheed, A., Shoukat, M.B., Rehman, A.U. 2021. Role of phyto-remediation in removing air pollutants: a review. *Cross Current International Journal of Agriculture and Veterinary Sciences,* **3:** 54-59.

Roy, A., Bhattacharya, T., Kumari, M. 2020. Air pollution tolerance, metal accumulation and dust capturing capacity of common tropical trees in commercial and industrial sites. *Science of The Total Environment*, **722:** 137622.

Sarhan, M.G., Abd Elhafeez, A., Bashandy, S. 2021. Evaluation of heavy metals concentration as affected by vehicular emission in alluvial soil at middle egypt conditions. *Egyptian Journal of Soil Science*, **61:** 337-354.

Schneidemesser, E.V., Stone, E. A., Quraishi, T.A., Shafer, M.M., Schauer, J.J. 2010. Toxic metals in the atmosphere in Lahore, Pakistan. *Science of The Total Environment*, **408:** 1640-1648.

Sharma, R.K., M. Agarwal. 2005. Biological effects of heavy metals: an overview. *Journal of Environmental Biology,* **26:** 301-313.

Shen, X., Dai, M., Yang, J., Sun, L., Tan, X., Peng, C., Ali, I., Naz, I. 2022. A critical review on the phyto-remediation of heavy metals from environment: performance and challenges. *Chemosphere*, **291:** 132979

Shirwani, R., Gulzar, S., Asim, M., Umair, M., Al-Rashid, M.A. 2020. Control of vehicular emission using innovative energy solutions comprising of hydrogen for transportation sector in Pakistan: a case study of Lahore city. *International Journal of Hydrogen Energy*, **45:** 16287-16297.

Singh, H. 2021. An integrated approach considering physiological and biophysical based indicators for assessing tolerance of roadside plantations of *Alstonia scholaris* towards urban roadside air pollution: an assessment of adaptation of plantations for mitigating roadside air pollution. *Trees, Springer Link on Published July 27, 2021.*

Tak, A., Kakde, U. 2019. Evaluation of trace elements and particulate matter deposition on plant foliage exposed to vehicular pollution. *Acta Botanica Croatica*, **78:** 164-168.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D.J. 2012. Heavy metal toxicity and the environment. *Experientia Supplementum*, **101:** 133-164.

Uka, U.N., Belford, E.J.D., Elebe, F.A. 2021. Effects of road traffic on photosynthetic pigments and heavy metal accumulation in tree species of *Kumasi metropolis*, Ghana. *SN Applied Sciences,* **3:** 131.

Wang, W., Zhang, M., Liu, J. 2018. Sub-cellular distribution and chemical forms of Cd in *Bougainvillea spectabilis* Willd. as an ornamental phyto-stabilizer: an integrated consideration. *International Journal of Phyto-remediation,* **20:** 1087-1095.

Wei, Z., Van Le, Q., Peng, W., Yang, Y., Yang, H., Gu, H., Lam, S.S., Sonne, C. 2021. A review on phyto-remediation of contaminants in air, water and soil. *Journal of Hazardous Materials*, **403:** 123658.

Yang, H., Zhang, X., Wang, G. 2004. Effects of heavy metals on stomatal movements in broad bean leaves. *Russian Journal of Plant Physiology*, **51:** 464-468.

Yang, L., Wang, J., Yang, Y., Li, S., Wang, T., Oleksak, P., Chrienova, Z., Wu, Q., Nepovimova, E., Zang, X., Kuca, K. 2022. Phyto-remediation of heavy metal pollution: hotspots and future prospects. *Ecotoxicology and Environmental Safety*, **234:** 113403.

Yaashikaa, P.R., Kumar, P.S., Jeevanantham, S., Saravanan, R. 2022. A review on bio-remediation approach for heavy metal detoxification and accumulation in plants. *Environmental Pollution*, **301:** 119035.