INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0478/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1525
http://www.fspublishers.org

Full Length Article

Physiological and Biochemical Responses of *Calotropis procera* to Traffic Related Cadmium Pollution along Roadside

Hina Batool[1*], Mumtaz Hussain[1], Mansoor Hameed[1] and Rashid Ahmad[2]
[1]*Department of Botany, University of Agriculture Faisalabad, 38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture Faisalabad, 38040, Pakistan*
[*]For correspondence: hussain.batool111@gmail.com
Received 25 March 2020; Accepted 15 May 2020; Published ________________

Abstract

The vehicles as a source of metal pollution have tremendous adverse effects on flora and fauna. Automobiles have recently increased the concentration of metal pollutants in urban areas and along the highway. This study determined the concentration of cadmium (Cd) released from the transport sector and its negative effects on *Calotropis procera* L. were determined along two major roads with diverse traffic volume *i.e*., Faisalabad to Gojra Motorway (M-4) and Gojra-Jhang Road (GJR) Punjab, Pakistan. Significant inhibitory changes were noted in the photosynthetic and transpiration rates of *C. procera* leaves along roads. Similarly, chlorophyll *a*, *b*, total chlorophyll and carotenoids were also significantly lower. Whereas, hydrogen peroxide, malondialdehyde, and total phenolic contents exhibited a stimulatory response. The Cd concentration (2.26 ± 0.40) in leaves of *C. procera* and soil (5.19 ± 0.30) remained higher along Gojra-Jhang road compared to Faisalabad-Gojra motorway. The Cd concentration was positively correlated indicative of having a strong contribution of vehicular traffic load. The correlation of different physiological and biochemical characteristics of plant with traffic load was significant that suggested the direct impact of vehicle exhaust on *C. procera* and showed the high tendency of plants to survive under metal-contaminated environment along roads. © 2020 Friends Science Publishers

Keywords: Cadmium; Photosynthetic pigments; Malondialdehyde; Phenolic contents; Traffic load

Introduction

The transport sector has become a major cause of metal pollution along the roadside (Mori *et al.* 2015) because it has adverse effects on plants and other living beings (Singh and Kalamdhad 2011; Showkat *et al*. 2019). Traffic released pollution influences public health as asthma, lung cancer and pulmonary related respiratory diseases (Marino *et al*. 2015; Kim *et al*. 2018). Traffic-related emissions include hydrocarbons, sulfur dioxide, particulate matter, nitrogen oxides and metal contaminants (Bhandarkar 2013; Deng *et al*. 2018).

These vehicles released pollutants are the primary contributor to pollution in the surrounding environment (Sujatha 2017) and are shown a tremendous impact on the flora and fauna (Khalid *et al.* 2017). Metal pollutants mainly enter the plants through root uptake mechanism. Plants consistently exposed to contamination and the brunt of automobile metal pollution by absorbing it at their foliar surface (Sarma *et al.* 2017; Khalid *et al.* 2018). These metals ultimate uptakes by plants and interrupts the food chain (Butt *et al.* 2018). Moreover, plants suffer deformities triggered by the metal pollutants and show metabolic changes. Hence due to this ability, plant proves as phytomonitor in the polluted environment and helps to attenuate the pollution (Cox 2003; Verma and Chandra 2014).

Cadmium (Cd) is a non-biodegradable and non-essential metal pollutant that adversely affects the metabolic process of plants even at its low level (Benavides *et al.* 2005). It is known as an effective contaminant due to its high toxicity and superior solubility in water (Orisakwe 2012). It is released from moving automobiles in and around the roadside (Adedeji *et al.* 2013). However, the unabated moving of large numbers of vehicles on roads can, therefore, lead to elevated levels of these pollutants in the plants and soil (Wekpe *et al.* 2019). These metallic Cd precipitated on the soil surrounding roads cause serious ecological hazards (Hashim *et al.* 2017). Li *et al.* (2018) found changes in physio-biochemical attributes of turnip leaves under Cd toxicity. Kapoor *et al.* (2014) also noted changes in physio-biochemical of *Brassica juncea* leaves under Cd stress. The Cd toxicity induced oxidative stress which damaged photosynthetic pigments and also causes physiological malfunctioning of plant (Irfan *et al.* 2013). Some plant species have been previously used for phytomonitoring of vehicular pollution along roadside include *Juglans regia*, *Cydonia oblanga*, and *Celtis australis* (Colak *et al.* 2016; Ozturk *et al.* 2017).

Of all the non-essential heavy metals, Cd is the metal which has attracted more attention in soil science and plant nutrition due to its potential toxicity to human beings, and also its relative movement in the soil-plant system. Therefore, the objectives were to determine the (1) spatial variation in Cd concentration in leaves of *C. procera* and soil along two roads (2) tremendous adverse effects on physiological and biochemical characteristics of most important bio-indicator plant species *Calotropis procera* (Aiton) W. T. Aiton commonly called apple of sodom.

Materials and Methods

Description of sites

Two roads *viz*., (1) a section Faisalabad-Gojra Motorway-4 (M-4) and (2) Gojra-Jhang Road (GJR) connecting Gojra to Jhang were selected for phytomonitoring of vehicular released Cd pollution and its effects on *C. procera* (Fig. 1). Eight sampling locations were selected on two roads at a distance of ~15 km between them. The roads varying in condition, traffic types and traffic volume. Motorway-4 newly constructed a section (58 km) of the road which directly joined Faisalabad to Gojra however, different types of field crops surrounds this section. Gojra headquarter linked to the Jhang district through Gojra-Jhang Road, the age of this road is about 100 years and have vehicle load about four times higher than that of M-4 road which is only four years old. Control site was also selected (50 m) away from the roads. The research project was conducted during the warm season (32°C).

Description of plant and soil samples

The *C. procera* plant was selected for phytomonitoring of metal pollution on the roadside. Triplicate matures leaves randomly were collected from the top, middle and bottom of the plant at each experimental site. Leaves surface was not washed after sampling to arrest all impurities. Three soil samples were collected from 0–10 cm depth at each study site. Control samples (leaves and soil) were also collected from 50 m away from the roads (Subramani and Devaanandan 2015; Khalid *et al.* 2018; Hadayat *et al.* 2019).

Samples digestion and analysis

Before the analysis the dried plant and soil samples (0.5 g) was processed using HNO_3/$HCLO_4$ acid digestion on the hot plate to determine their cadmium (Cd) concentration (USEPA 1996). Atomic absorption spectrophotometer (Hitachi, 8200 Japan) was used to determine Cd concentration. 1,000 mg/L Cd stock solution was used for calibration and quality assurance. Standard and blank were analyzed after every 20 samples to monitor the stability of the apparatus. Highly pure acids and distilled H_2O used for making a blank sample.

Gas exchange parameters

Transpiration (*E*) and photosynthetic (*A*) rates were noted from the leaves of *C. procera* using infrared gas analyzer (LCA-4, Portable Analytical Development Company (ADC), Hoddeson, England).

Chlorophyll contents

The chlorophyll and carotenoid pigments were determined by using the protocol (Arnon 1949; Davis 1979) respectively. The chlorophyll from leaf sample was extracted with pre-cooled 80% acetone solution. The leaf extract was centrifuged (1000 rpm) and separated the supernatant. Spectrophotometer model IRMECO U2020 was used to measure the optical density at wavelengths 663, 645 and 480 nm.

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) was estimated by the TCA reaction (Velikova *et al.* 2000). The leaves were immediately frozen in liquid nitrogen (-210°C) and directly homogenized with trichloroacetic acid (TCA). After centrifugation, the resulting supernatant was rapidly mixed with phosphate buffer 0.5 mL and 1 mL potassium iodide solution. Samples were left to incubate at room temperature. The absorbance was taken at 390 nm on a spectrophotometer. A standard curve obtained with H_2O_2 standard solutions depicted as μmol/g.

Malondialdehyde (MDA)

The MDA content was measured in leaf sample extracted with trichloroacetic acid (TCA). After centrifugation, the separated supernatant (1 mL) was mixed with (2.5 mL) thiobarbituric acid (TBA) with TCA and incubated in a water bath (Ali *et al*. 2005). Subsequently, cooled the mixture immediately and the optical density was noted at 532 and 600 nm on a spectrophotometer. The MDA concentration was measured by the following formula, using an absorbance coefficient of extinction (155000 nmol mL^{-1}) as.

$$MDA= [A532-A600)/155000] \times 10^6$$

Total phenolic contents (TPC)

The fresh leaf sample was extracted with 80% aqueous acetone solution. The supernatant (100 μL) was mixed immediately with Folin-Ciocalteu reagent 0.5 mL and added 2 mL of 20% sodium carbonate solution (Julkunen-Tiitto 1985). Optical density was taken at 750 nm on a spectrophotometer. The total phenolic content was depicted as mg of gallic acid/g sample.

$$TPC = C/Vm$$

Traffic density

Traffic density was noted to correlate it Cd concentration accumulated in the plant and the soil from toll plaza present on M-4 and GJR roads.

Statistical analysis

The data were analyzed by two-way ANOVA (Statistix 8.0). For means comparison, LSD (5%) test was used. Pearson's correlation coefficient was calculated between Cd concentration and traffic density. Average and standard error values were calculated for graphical presentation by using MS-Excel 2016.

Results

The Cd pollution from vehicles origin varied significantly ($P < 0.001$) both in leaves of *C. procera* and in the soil of different sites along M-4 and GJR roads (Table 1). Two-way analysis (ANOVA) revealed significant variations for Cd concentration between roads (F = 249.1, $P < 0.001$; F = 3107.9, $P < 0.001$) and among sites (F =82.59, $P < 0.001$; F = 1390.5, $P < 0.001$) both in leaves of *C. procera* and soil respectively (Table 1 and Fig. 2a). The higher Cd concentration was accumulated along the roadside compared to control. The leaves and soil along GJR road trapped the highest concentration of Cd compared to M-4 road (Fig. 2a). The higher traffic volume was recorded on GJR road compared to M-4 road (Fig. 2b). The Cd concentration in the roadside plant leaves and the soil were higher at Gojra Interchange (GI) site along with M-4 road and Forest Park (FP) site on GJR road. Strong positive correlation ($P < 0.1$) was observed between Cd concentration accumulated in the soil and traffic density. Also, strong positive correlation (M-4, $P < 0.1$; GJR, $P < 0.05$) was found between Cd concentration get accumulated in leaves of *C. procera* and traffic volume (Table 2).

According to ANOVA, a significant reduction (roads, $P < 0.001$; sites, $P < 0.001$) was observed in photosynthetic and transpiration rates of *C. procera* leaves at various experimental sites along two roads (Table 1). Along M-4, the highest reduction in photosynthetic (7.71 ± 0.44) and transpiration rates (1.09 ± 0.07) was recorded at Gojra Interchange (GI) site. The highest reduction in photosynthetic rate (2.63 ± 0.28) and transpiration rate (0.53 ± 0.07) was noted at Forest Park (FP) site on GJR road (Fig. 3). Strong negative correlation was analyzed between gas exchange attributes as photosynthetic rate (r = -0.94, $P < 0.05$; r = -0.91, $P < 0.05$) and transpiration rate (r = -0.781 ns; r = -0.93, $P < 0.05$) in *C. procera* and traffic volume

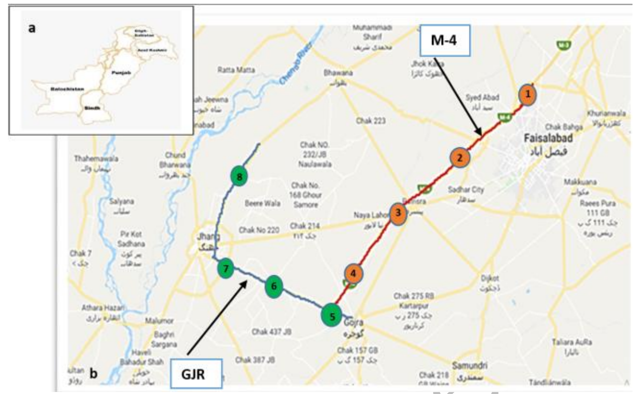

Fig. 1: Map showing **a**). Pakistan **b**). Sites along Faisalabad-Gojra Motorway (M-4) and Gojra-Jhang Road (GJR) roads where, (1) Sargodha Road Intergange (SRI); (2) Amin Pur Interchange (API); (3) Pansara Interchange (PI);(4) Gojra Interchange (GI); (5) Boback Chowk (BC); (6) Chak Adda (CA); (7) Forest Park (FP); (8) Shabir Abad (SA).

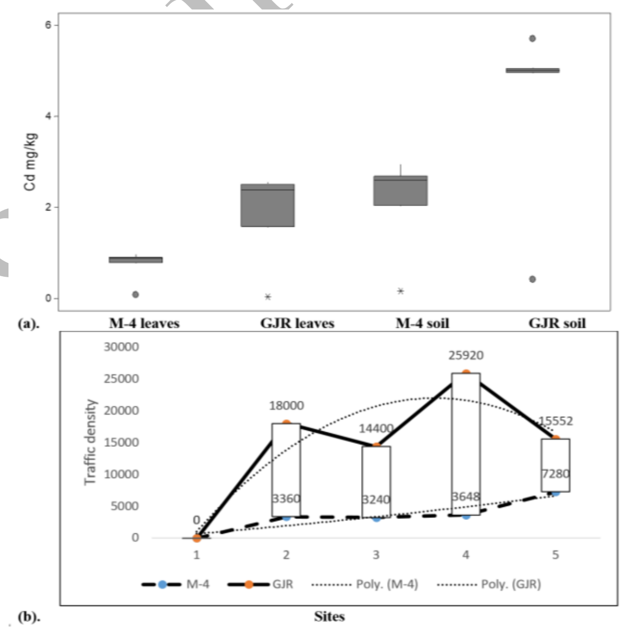

Fig. 2: (**a**). Box and whisker plot for Cd concentrations in *C. procera* leaves and in the soil along M-4 and GJR roads. (**b**). Traffic density (no. of vehicles per/day) at various sites on M-4 and GJR roads

along M-4 and GJR roads respectively (Table 2).

The highly significant reduction between roads (F = 38.70, $P < 0.001$; F =15.95, $P < 0.001$; F = 66.23, $P < 0.001$; F = 30.24, $P < 0.001$) and among sites (F = 173.7, $P < 0.001$; F = 88.68, $P < 0.001$; F = 313.4, $P < 0.001$; F = 25.76, $P < 0.001$) was noted for chlorophyll *a, b,* total chlorophyll and carotenoids respectively on both roads (Table 1). Along GJR road, chlorophyll *a, b,* total chlorophyll and carotenoid contents in leaves of *C. procera* were significantly lower (Fig. 4a–d). However, along GJR

Table 1: Two way-ANOVA (F-ratio) for cadmium (Cd) concentration (mg/kg) in leaves, soil and phytochemicals in leaves of *C. procera* along both M-4 and GJR roads

Source	Cd-Leaves (mg kg^{-1})	Cd-Soil (mg kg^{-1})	Chl. *a* (mg g^{-1} f. wt.)	Chl. *b* (mg g^{-1} f. wt.)	T. Chl. (mg g^{-1} f. wt.)	Car. (mg g^{-1} f. wt.)	H_2O_2 (μmol g^{-1} f. wt.)	MDA (mmolml^{-1} f. wt.)	TPC (mg g^{-1} f. wt.)	*A* (μmol CO_2 m^{-2} S^{-1})	*E* (mmol H_2O m^{-2} S^{-1})
Road	249.1***	3107.6***	38.70***	15.95***	66.23***	30.24***	0.11ns	2.03ns	18.85 ***	133.05***	18.72***
Site	82.59***	1390.5***	173.7***	88.68***	313.4***	25.76***	49.10***	2.92*	28.58 ***	34.50***	40.72***
Road×site	25.13***	203.8***	29.49***	3.601*	31.97***	6.759**	7.76***	2.95 *	9.052***	9.64***	1.58ns

Non-significant = ns, significant * = $P < 0.05$, significant ** = $P < 0.01$, significant *** = $P < 0.001$. Attributes presented as Cd-Leaves; cadmium concentration in leaves, Cd-Soil; cadmium concentration in soil, Chl. *a*; chlorophyll *a*, Chl. *b*; chlorophyll *b*, T. Chl.; total chlorophyll, Car.; carotenoids, TPC; total phenolics content, MDA; malondialdehyde, H_2O_2; hydrogen peroxide

Table 2: Correlation of traffic volume on M-4 and GJR roads with Cd content and phytochemicals

Traffic volume	Cd-leaves	Cd-soil	Chl. *a*	Chl. *b*	T. Chl.	Car.	H_2O_2	MDA	TPC	*A*	*E*
M-4	0.834*	0.857*	-0.923**	-0.767ns	-0.875*	-0.897**	0.844*	0.799ns	0.845*	-0.948**	-0.781ns
GJR	0.880**	0.833*	-0.948**	-0.744ns	-0.878*	-0.474ns	0.729ns	0.900**	0.815*	-0.917**	-0.932**

Significant * = $P < 0.1$, significant ** = $P < 0.05$. Attributes presented as Cd-Leaves; cadmium concentration in leaves, Cd-Soil; cadmium concentration in soil, Chl. *a*; chlorophyll *a*, Chl. *b*; chlorophyll *b*, T. Chl.; total chlorophyll, Car.; carotenoids, TPC; total phenolics content, MDA; malondialdehyde, H_2O_2; hydrogen peroxide

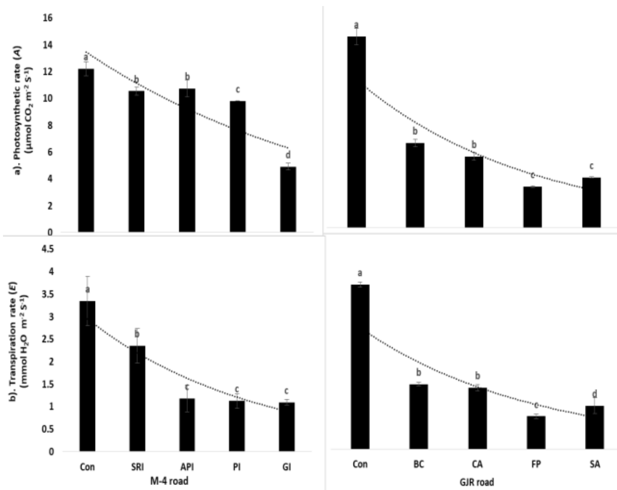

Fig. 3: Gas exchange parameters **a**). Photosynthetic rate (*A*) **b**). Transpiration rate (*E*) at various sites on M-4 and GJR roads. Abbreviations: Faisalabad-Gojra Motorway (M-4) and Gojra-Jhang Road (GJR) roads where, Control (Con), Sargodha Road Intergange (SRI), Amin Pur Interchange (API), Pansara Interchange (PI), Gojra Interchange (GI), Boback Chowk (BC), Chak Adda (CA), Forest Park (FP), Shabir Abad (SA)

road minimum chlorophyll *a* (0.314 ± 0.000), *b* (0.287 ± 0.004) and total chlorophyll (0.602 ± 0.004) were recorded at the Forest Park (FP) site whereas, carotenoid contents (0.690 ± 0.129) was lower at the Chak Aadda (CA) site (Fig. 4a–d). Of all the studied sites along M-4 road, the higher reduction in chlorophyll *a* (0.359 ± 0.03), *b* (0.437 ± 0.046), total chlorophyll (0.797 ± 0.083) and carotenoid contents (0.545 ± 0.08) were found at the Gojra Interchange (GI) site (Fig 4). However, a strong negative correlation was found between traffic volume and chlorophyll a (for both roads $P < 0.05$), chlorophyll b (M-4, GJR=ns), total chlorophyll (M-4, $P < 0.1$; GJR, $P < 0.05$) and carotenoid contents (M-4, $P < 0.05$; GJR=ns) (Table 2).

Due to Cd toxicity, the levels of ROS increased which damaged the membranes of cellular organelles and

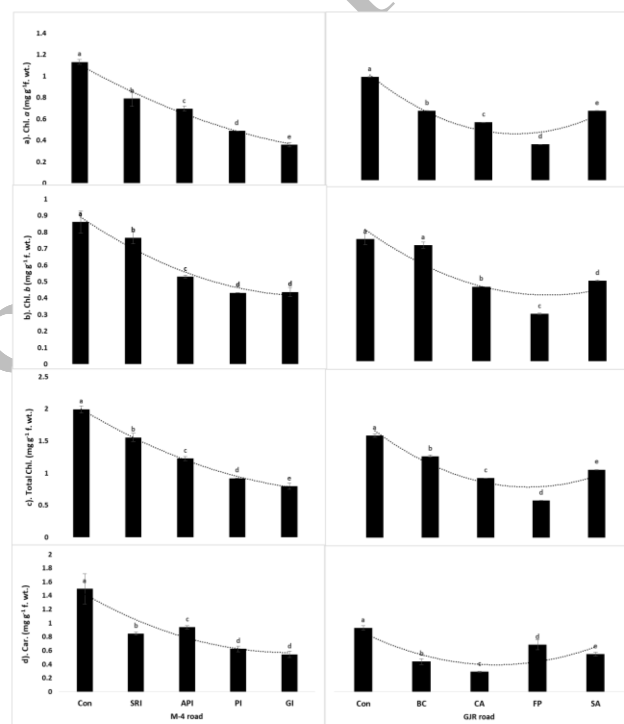

Fig. 4: Photosynthetic pigments **a**). Chlorophyll *a* (Chl. *a*), **b**). Chlorophyll *b* (Chl. *b*) **c**). Total chlorophyll (T. Chl.) and **d**). Carotenoids (Car.) at various sites on M-4 and GJR roads. Abbreviations: Faisalabad-Gojra Motorway (M-4) and Gojra-Jhang Road (GJR) roads where, Control (Con), Sargodha Road Intergange (SRI), Amin Pur Interchange (API), Pansara Interchange (PI), Gojra Interchange (GI), Boback Chowk (BC), Chak Adda (CA), Forest Park (FP), Shabir Abad (SA)

enhanced the accumulation of malondialdehyde byproduct in leaves of *C. procera*. In this study, we analyzed the localization of hydrogen peroxide in leaves of *C. procera* growing near the roads (Fig. 5b). The analysis of variance showed insignificant variation for hydrogen peroxide between roads (F =0.108, ns) but we found significant variation among sites (F = 49.10, $P < 0.001$). Whereas, the

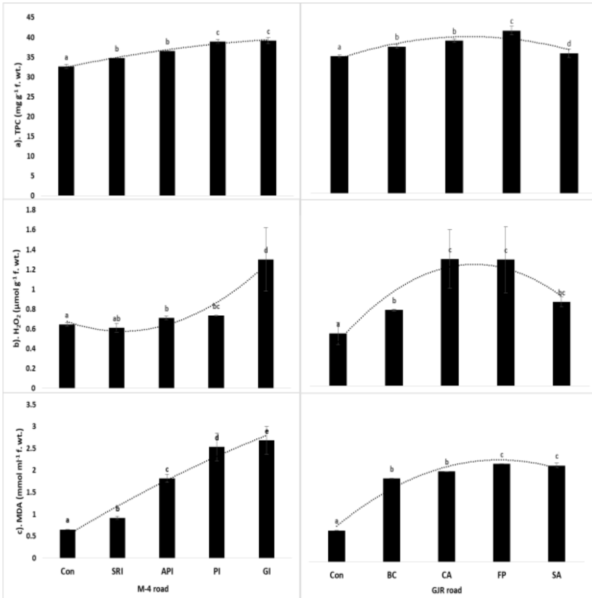

Fig. 5: Oxidative stress and antioxidant **a**). Total phenolics content (TPC), **b**). Hydrogen peroxide (H_2O_2) **c**). Malondialdehyde (MDA) at various sites on M-4 and GJR roads. Abbreviations: Faisalabad-Gojra Motorway (M-4) and Gojra-Jhang Road (GJR) roads where, Control (Con), Sargodha Road Intergange (SRI), Amin Pur Interchange (API), Pansara Interchange (PI), Gojra Interchange (GI), Boback Chowk (BC), Chak Adda (CA), Forest Park (FP), Shabir Abad (SA)

level of malondialdehyde also showed insignificant differences for roads (F = 2.030, ns), and significant among sites (F = 2.916, $P < 0.05$, Fig. 5c). Due to oxidative state in leaves of *C. procera* total phenolic contents was significantly higher (Table 1). The maximum accumulation of hydrogen peroxide (2.684 ± 0.551) was noted at the Gojra Interchange (GI) site on M-4. Along GJR the highest level of hydrogen peroxide (2.149 ± 0.003) in *C. procera* leaves (Fig. 5b). The highest malondialdehyde accumulation was seen at Gojra Interchange (GI) (1.30 ± 0.551) and Chak Aadda (CA) (1.31 ± 0.527) on M-4 and GJR roads respectively.

The non-enzymatic antioxidant as total phenolic contents (39.20 ± 1.34; 41.98 ± 1.88) was found the maximum at the Gojra Interchange (GI) and Forest Park (FP) sites along both M-4 and GJR roads respectively (Fig. 5a). There was also a positive correlation with traffic density for total phenolics contents (M-4, GJR=$P < 0.1$), malondialdehyde (M-4, $P < 0.1$; GJR=ns) and hydrogen peroxide (M-4=ns; GJR, $P < 0.05$) (Table 2).

Discussion

Plant respond to environmental stress and environmental pollution is leading factor that exerted stress on plant. In this regard, physio-biochemical characteristics such as photosynthetic, transpiration rates, photosynthetic pigments, hydrogen peroxide, malondialdehyde and total phenolic contents are used as reliable indicators of Cd toxicity in plants. Vehicles as a remarkable source of Cd pollutants near the road environment (Zheng 2017) which drastically changed the physio-biochemical characteristics of flora (Verma and Chandra 2014) and can deposit more metal concentration in the soil through foliar action (Modrzewska and Wyszkowski 2014). Moreover, Cd emission along the roadside is mainly from lubricants and greases used in vehicles and tire wear (Massadeh *et al.* 2004).

The extent of Cd was noted above the allowable limits recommended by the World Health Organization (WHO) for plants (0.02 mg/kg) and according to the Dutch Standard *i.e.*, 0.8 mg/kg for soil (WHO 1996). Ogundele *et al.* (2015) found Cd pollutants in plants and soil of various sites between (0.028–4.00 mg/kg) and (0.00–0.366 mg/kg) respectively. This may be due to heavy traffic running on both roads which continuously, distributes the Cd toxicity in roadside soil (Zhang *et al.* 2012; Colak *et al.* 2016) and plants get accumulate it both through their uptake (Ismael *et al.* 2019) and foliar mechanisms deposit on the plant leaf surface (Shahid *et al.* 2017; Sulaiman and Hamzah 2018). The metal contaminants come from various types of activities related to the automobile on roads. For example, abrasion of body parts, wear and tear of tires and vehicles paints etc. (Adamiec *et al.* 2016). The greater concentration along GJR road compared to M-4 might be related to heavy traffic, uneven road surface and types of vehicles and age of road (~100 years), of GJR. As the age of road has directly linked with metal pollution (Wang and Zhang 2018).

The metals deposited in the soil near roadside suggest the contribution of traffic volume (Wang and Zhang 2018; Szwalec *et al.* 2020). Likely, metal accumulation differs depending upon traffic volume in some plants have been reported in *C. procera* (Tiwari 2016; Hadayat *et al.* 2019) and *Tilia tomentosa* (Turkyilmaz *et al.* 2018). Several previous reports are available which indicate the Cd concentration was high near the road edge (Krailertrattanachai *et al.* 2019). The Cd concentrations found in *C. procera* plant and roadside soil were affected by the vehicular load. The primary source of metal contamination was strongly associated with automobiles (Adedeji *et al.* 2013; Rozanski *et al.* 2017; Ahmad *et al.* 2018; Khalid *et al.* 2018).

Pearson correlation indicated that Cd contamination was highly correlated with vehicle density of two roads. Previous studies also reported a significant association between metal concentration and vehicles density (Hu *et al.* 2018). Some scientists also found a significant association between metal ions and the number of passing vehicles (Arslan 2001; Alhassan *et al.* 2012). Sulaiman and Hamzah (2018) reported a positive association of metal concentration with traffic load in Malaysia. In this study the physio-phytochemical response of *C. procera* plant under the influence of Cd toxicity related to vehicular emission along both roads was also observed. During this phytomonitoring, a decrease in gas exchange features *i.e*., photosynthetic and transpiration rates of *C. procera* was found. Some findings in line with the previous researchers, for example, Nawazish *et al.* (2012) reported transpiration and photosynthetic rates decreased in various wild plant species due to metal toxicity along the roadside. Likewise, Hadayat *et al.* (2019) noted the decreased trend in gas exchange attributes *i.e*., photosynthetic and transpiration rates of plant due to roadside contamination.

The photosynthetic rate decreased at the roadside because metal pollutants interact with metabolic processes in the leaves of plants (Nawazish *et al.* 2012). These metal pollutants released from automobiles block the stomata, leading to a shortage of carbon dioxide (CO_2) thus it arrests photosynthetic carbon assimilation and reduces transpiration rate in the plants (Anjum *et al.* 2016). The general reduction of physiological attributes was noted in various plant species as a response of vehicular released pollution on roads *S. Japonica* (Bao *et al.* 2015), *Cenchrus ciliaris* (Nawazish *et al.* 2012), and *L. speciose* (Singh *et al.* 2017). The Cd toxicity adversely affected chlorophyll pigments along the roadside compared to a reference plant in this investigation. Photosynthesis is greatly sensitive to any changes in the environment and the reduction of chlorophyll content has a direct link with the growth and vigor of the plant (Kalaji *et al.* 2018). The main target of Cd toxicity is the inhibition of enzymes involved in photosynthesis (Joshi and Mohanty 2004; Hassan *et al*. 2016; Song *et al.* 2019). However, the metal pollutants may destroy chloroplast apparatus by interfering with photosynthetic enzymes and damage the membrane of the chloroplast and therefore can inhibit the photosynthetic process in the plant leaves (Parmar *et al.* 2013). Considerable decrease in chlorophyll pigments of some plant species, for example, *Urginea maritima* (Houri *et al*. 2019) and *Mangifera indica* (Uka *et al.* 2019), due to the roadside vehicular metal pollution has been already noted. Previous work supported present study results that chlorophyll contents of various roadside plants were affected with automobiles emission (Iqbal *et al.* 2015; Shiragave *et al*. 2015).

Oxidative stress occurs in response to metal pollution and releases the ROS in plant tissues *i.e*., hydrogen peroxide, singlet oxygen, hydroxyl radical, *etc*. which is one of the important responses of the plant to stress condition. So, boost the production of these species from metal toxicity causes oxidative stress (Kohli *et al.* 2017). ROS play a role as a secondary messenger in various cellular activities (Berni *et al.* 2019). Higher levels of hydrogen peroxide along both M-4 and GJR roads were found. Cells exposed to oxidative stress show toxicity symptoms. This is because of the interaction with ROS (Shahid *et al.* 2014). Under metal pollution production of ROS results in lipid peroxidation, damages to protein, DNA and carbohydrates. A well-known secondary product like MDA of lipid peroxidation due to stress of ROS (Muszynska *et al.* 2019). Significant production of MDA content was recorded in the metal treated plants, indicative of maximal cell membrane damage (Alfanie *et al.* 2015). As the antioxidants in plants play a defense role in oxidative stress under metal pollution (Afzal *et al.* 2014). These defense systems work jointly to control the oxidative stress (Shahid *et al.* 2014; Sharma *et al.* 2019).

Recently, it has been examined that flavonoids, phenylopropanoids and phenolic acids have a potential role in the antioxidative ability of plants as compared to other antioxidants (Michalak 2006). Phenolic compounds acknowledged as metal chelators when exposed to heavy metals. Moreover, phenolics directly scavenge ROS. Phenolics compounds have multiple roles in respect to an adaptive measure of plants to the environmental stress (Boscaiu *et al.* 2010). Phenolics are the most important secondary metabolites and involved in the physiological process of plants (Kumar *et al.* 2019). In present work, the phenolic contents were enhanced in *C. procera* growing around both roads under Cd toxicity. The finding supports our results, for example, Olivares (2003) found the increased level of phenolics compound in *Tithonia diversifolia* due to metal toxicity along the roadside.

The significant negative correlation found between photosynthetic, transpiration rates, chlorophyll a, total chlorophyll and carotenoids, with traffic volume along both of the studied roads indicated that increase the negative impacts on *C. procera* probably due to vehicles emission on roads. Similarly, the positive correlation between hydrogen peroxide, malondialdehyde and phenolics contents with traffic volume on both roads. However, the insignificant correlations between various physio-biochemical characteristics of *C. procera* with traffic density existed on M-4 and GJR might be due to a slight difference in traffic volume resulting to same or different metal concentration at sites over there (Khalid *et al.* 2017).

Conclusion

In this study increased level of Cd pollutants were noted in *C. procera* leaves and soil along M-4 and GJR roads. Due to the Cd toxicity, inhibitory effects were noted in photosynthetic pigments, photosynthetic and transpiration rates with an elevated level of hydrogen peroxide, malondialdehyde as well as phenolic contents in *C. procera* at several different sites proximal to the roads. The results show that roadside Cd pollution induces changes in physio-biochemical aspects of *C. procera* which helps it in maintaining stressed conditions, thus, supporting its hyper-accumulating potential.

Acknowledgments

The work presented here was conducted by the author Ms. Hina Batool for the fulfillment of her PhD. (Botany) Dissertation. Also, acknowledged the Central High-Tech Lab of Agriculture University, Faisalabad, Pakistan for analysis of heavy metal.

Author Contributions

Hina Batool executed the research and made the write-up, Mumtaz Hussain planned the research and analyzed the data, Mansoor Hameed reviewed the article, Rashid Ahmed planned the research.

References

Adamiec E, E Jarosz-Krzeminska, R Wieszala (2016). Heavy metals from non-exhaust vehicle emissions in urban and motorway road dusts. *Environ Monit Assess* 188:369–379

Adedeji OH, OO Olayinka, FF Oyebanji (2013). Assessment of traffic related heavy metals pollution of roadside soils in emerging urban centres in Ijebu-north area of Ogun State, Nigeria. *J Appl Sci Environ Manage* 17:509–514

Afzal F, R Khurshid, M Ashraf, AG Kazi (2014). Reactive oxygen species and antioxidants in response to pathogens and wounding. *In: Oxidative Damage to Plants*, pp:397–424. Academic Press

Ahmad I, M Hussain, M Hameed, R Ahmad (2018). Adverse effects of automobiles related Pb^+ pollution on photosynthetic attributes and water relations of roadside vegetation. *Pak J Bot* 50:517–527

Alfanie I, R Muhyi, E Suhartono (2015). Effect of heavy metal on malondialdehyde and advanced oxidation protein products concentration a focus on arsenic, cadmium, and mercury. *J Med Bioeng* 4:1–6

Alhassan AJ, MS Sule, MK Atiku, AM Wudil, MA Dangambo, JA Mashi, NA Ibrahim (2012). Study of correlation between heavy metal concentration, street dust and level of traffic in major roads of Kano Metropolis, Nigeria. *Nig J Basic Appl Sci* 20:161–168

Ali MB, EJ Hahn, KY Paek (2005). Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micro-propagated Phalaenopsis plantlet. *Environ Exp Bot* 54:109–120

Anjum SA, U Ashraf, I Khan, M Tanveer, MF Saleem, L Wang (2016). Aluminum and chromium toxicity in maize, implications for agronomic attributes, net photosynthesis, physio-biochemical oscillations, and metal accumulation in different plant parts. *Water Air Soil Pollut* 227:326

Arnon DI (1949). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris. Plant Physiol* 24:1–15

Arslan H (2001). Heavy metals in street dust in Bursa, Turkey. *J Trace Microprob Tech* 19:439–445

Bao L, K Ma, S Zhang, L Lin, L Qu (2015). Urban dust load impact on gas-exchange parameters and growth of *Sophora japonica* L. seedlings. *Plant Soil Environ* 61:309–315

Benavides MP, SM Gallego, ML Tomaro (2005). Cadmium toxicity in plants. *Braz J Plant Physiol* 17:21–34

Berni R, M Luyckx, X Xu, S Legay, K Sergeant, JF Hausman, G Guerriero (2019). Reactive oxygen species and heavy metal stress in plants impact on the cell wall and secondary metabolism. *Environ Exp Bot* 161:98–106

Bhandarkar S (2013). Vehicular pollution, their effect on human health and mitigation measures. *Vehicle Eng* 1:33–40

Boscaiu M, M Sanchez, I Bautista, P Donat, A Lidon, J Llinares, O Vicente (2010). Phenolic compounds as stress markers in plants from gypsum habitats. *Bull UASVM Hortic* 67:44–49

Butt A, Qurat-ul-Ain, K Rehman, MX Khan, T Hesselberg (2018). Bioaccumulation of cadmium, lead, and zinc in agriculture-based insect food chains. *Environ Monit Assess* 190:698-709

Colak M, M Gumrukçuoglu, F Boysan, E Baysal (2016). Determination and mapping of cadmium accumulation in plant leaves on the highway roadside, Turkey. *Arch Environ Prot* 42:11–16

Cox RM (2003). The use of passive sampling to monitor forest exposure to O_3, NO_2 and SO_2: A review and some case studies. *Environ Pollut* 126:301–311

Davis BH (1979). *Chemistry and Biochemistry of Plant Pigments*, 2nd edn, pp:149–155. Godwin TW (Ed.). Academic Press Inc., London

Deng C, Y Jin M Zhang X Liu, Z Yu (2018). Emission characteristics of VOCs from on road vehicles in an urban tunnel in Eastern China and predictions for 2017–2026. *Aerosol Air Qual Res* 18:3025–3034

Hadayat N, M Hussain, M Shahbaz, R Ahmad (2019). Physio-biochemical responses of apple-of-sodom [*Calotropis procera* (Aiton) W.T. Aiton] to vehicular pollution. *Pak J Bot* 51:609–616

Hashim T, HH Abbas, IM Farid, O El-Husseiny, MH Abbas (2017). Accumulation of some heavy metals in plants and soils adjacent to Cairo–Alexandria agricultural highway. *Egypt J Soil Sci* 57:215–232

Hassan W, S Bashir, F Ali, M Ijaz, M Hussain, J David (2016). Role of ACC-deaminase and/or nitrogen fixing rhizobacteria in growth promotion of wheat (*Triticum aestivum* L.) under cadmium pollution. *Environ Earth Sci* 75:267

Houri T, Y Khairallah, A Al-Zahab, B Osta, D Romanos, G Haddad (2019). Heavy metals accumulation effects on the photosynthetic performance of geophytes in Mediterranean reserve. *J King Saud Univ Sci* 32:874-880

Hu R, Y Yan, X Zhou, Y Wang, Y Fang (2018). Monitoring heavy metal contents with *Sphagnum junghuhnianum* moss bags in relation to traffic volume in Wuxi, China. *Intl J Environ Res Publ Health* 15:374-385

Iqbal M, M Shafiq, S Zaidi, M Athar (2015). Effect of automobile pollution on chlorophyll content of roadside urban trees. *Glob J Environ Sci Manage* 1:283–296

Irfan M, S Hayat, A Ahmad, MN Alyemeni (2013). Soil cadmium enrichment allocation and plant physiological manifestations. *Saud J Biol Sci* 20:1–10

Ismael MA, AM Elyamine, MG Moussa, M Cai, X Zhao, C Hu (2019). Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics* 11:255–277

Joshi MK, P Mohanty (2004). Chlorophyll a fluorescence as a probe of heavy metal ion toxicity in plants. *In*: *Chlorophyll a Fluorescence*, pp:637–661. Springer, Dordrecht, The Netherlands

Julkunen-Tiitto R (1985). Phenolic constituents in the leaves of northern willows methods for the analysis of certain phenolics. *J Agric Food Chem* 33:213–217

Kalaji HM, L Rackova, V Paganova, T Swoczyna, S Rusinowski, K Sitko (2018). Can chlorophyll-a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? *Environ Exp Bot* 152:149–157

Kapoor D, S Kaur, R Bhardwaj (2014). Physiological and biochemical changes in *Brassica juncea* plants under Cd-induced stress. *BioMed Res Intl* 2014:1-13

Khalid N, M Hussain, HS Young, B Boyce, M Aqeel, A Noman (2018). Effects of road proximity on heavy metal concentrations in soils and common roadside plants in Southern California. *Environ Sci Pollut Res* 25:35257–35265

Khalid N, M Hussain, M Hameed, R Ahmad (2017). Physiological, biochemical and defense system responses of *Parthenium hysterophorus* to vehicular exhaust pollution. *Pak J Bot* 49:67–75

Kim D, Z Chen, LF Zhou, SX Huang (2018). Air pollutants and early origins of respiratory diseases. *Chron Dis Translat Med* 4:75–94

Kohli SK, N Handa, V Gautam, S Bali, A Sharma, K Khanna, YE Kolupaev (2017). ROS signaling in plants under heavy metal stress. *In: Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*, pp:185–214. Springer, Singapore

Krailertrattanachai N, D Ketrot, W Wisawapipat (2019). The distribution of trace metals in roadside agricultural soils, Thailand. *Intl J Environ Res Publ Health* 16:714-725

Kumar V, A Sharma, SK Kohli, S Bali, M Sharma, R Kumar, AK Thukral (2019). Differential distribution of polyphenols in plants using multivariate techniques. *Biotechnol Res Innovat* 3:1–21

Li X, X Zhang, Y Wu, B Li, Y Yang (2018). Physiological and biochemical analysis of mechanisms underlying cadmium tolerance and accumulation in turnip. *Plant Divers* 40:19–27

Marino E, M Caruso, D Campagna, R Polosa (2015). Impact of air quality on lung health: Myth or reality? *Therapeut Adv Chron Dis* 6:286–298

Massadeh AM, M Tahat, QM Jaradat, IF Al-Momani (2004). Lead and cadmium contamination in roadside soils in Irbid City, Jordan: A case study. *Soil Sedim Contaminat* 13:347–359

Michalak A (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 15:523–530

Modrzewska B, M Wyszkowski (2014). Trace metals content in soils along the state road 51 (northeastern Poland). *Environ Monit Assess* 186:2589–2597

Mori J, HM Hanslin, G Burchi, A Saebo (2015). Particulate matter and element accumulation on coniferous trees at different distances from a highway. *Urban For Urban Green* 14:170–177

Muszynska E, M Labudda, E Rozanska, E Hanus-Fajerska, A Koszelnik-Leszek (2019). Structural, physiological and genetic diversification of *Silene vulgaris* ecotypes from heavy metal-contaminated areas and their synchronous *in vitro* cultivation. *Planta* 249:1761–1778

Nawazish S, M Hussain, M Ashraf, MY Ashraf, A Jamil (2012). Effect of automobile related metal pollution (Pb^{2+} and Cd^{2+}) on some physiological attributes of wild plants. *Intl J Agric Biol* 14:953–958

Ogundele DT, AA Adio, OE Oludele (2015). Heavy metal concentrations in plants and soils along heavy traffic roads in North Central Nigeria. *J Environ Anal Toxicol* 5:1–5

Olivares E (2003). The effect of lead on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution or grown in pots of Pb-supplemented soil. *Braz J Plant Physiol* 15:149–158

Orisakwe OE (2012). Other heavy metals: Antimony, cadmium, chromium and mercury. *In: Toxicity Building Mater*, pp:297–333. Woodhead Publishing, Cambridge, UK

Ozturk A, C Yarci, II Ozyigit (2017). Assessment of heavy metal pollution in Istanbul using plant (*Celtis australis* L.) and soils assays. *Biotechnol Biotechnol Equip* 31:948–954

Parmar P, N Kumari, V Sharma (2013). Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Bot Stud* 54:45-50

Rozanski S, H Jaworska, K Matuszczak, J Nowak, A Hardy (2017). Impact of highway traffic and the acoustic screen on the content and spatial distribution of heavy metals in soils. *Environ Sci Pollut Res* 24:12778–12786

Sarma B, SK Chanda, M Bhuyan (2017). Impact of dust accumulation on three roadside plants and their adaptive responses at National Highway 37, Assam, India. *Trop Plant Res* 4:161–167

Shahid M, C Dumat, S Khalid, E Schreck, T Xiong, NK Niazi (2017). Foliar heavy metal uptake, toxicity and detoxification in plants a comparison of foliar and root metal uptake. *J Hazard Mater* 325:36–58

Shahid M, B Pourrut, C Dumat, M Nadeem, M Aslam, E Pinelli (2014). Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. *Rev Environ Contam Toxicol* 232:1-44

Sharma A, B Shahzad, V Kumar, SK Kohli, GPS Sidhu, AS Bali, N Handa, D Kapoor, R Bhardwaj, B Zheng (2019). Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules* 9:285-320

Shiragave PD, AA Ramteke, SD Patil (2015). Plant responses to vehicular pollution: Specific effect on photosynthetic pigments of plants at divider of NH-4 highway Nipani Area, Karnataka State, India. *Young* 139:122–138

Showkat A, T Hassan, S Majid (2019). Heavy metal toxicity and their harmful effects on living organisms a-review. *Intl J Med Sci Diagn Res* 3:106–122

Singh H, R Sharma, S Sinha, M Kumar, P Kumar, A Verma, SK Sharma (2017). Physiological functioning of *Lagerstroemia speciosa* L. under heavy roadside traffic, an approach to screen potential species for abatement of urban air pollution. *3Biotech* 7:61–68

Singh J, AS Kalamdhad (2011). Effects of heavy metals on soils, plants, human health and aquatic life. *Intl J Res Chem Environ* 1:15–21

Song X, X Yue, W Chen, H Jiang, Y Han, X Li (2019). Detection of cadmium risk to the photosynthetic performance of hybrid *Pennisetum*. *Front Plant Sci* 10; Article 798

Subramani S, S Devaanandan (2015). Application of air pollution tolerance index in assessing the air quality. *Intl J Pharm Pharm Sci* 7:216–221

Sujatha P (2017). Contribution of vehicular emissions on urban air quality: Results from public strike in Hyderabad. *Ind J Radio Space Phys* 43:340–348

Sulaiman FR, HA Hamzah (2018). Heavy metals accumulation in suburban roadside plants of a tropical area (Jengka, Malaysia). *Ecol Proc* 7:28-38

Szwalec A, P Mundala, R Kędzior, J Pawlik (2020). Monitoring and assessment of cadmium, lead, zinc and copper concentrations in arable roadside soils in terms of different traffic conditions. *Environ Monit Assess* 192:155-166

Tiwari S (2016). Biomonitoring of toxic metals through roadside vegetation exposed to vehicular pollution in Bilaspur city. *Environ Skept Crit* 5:57–62

Turkyilmaz A, H Sevik, M Cetin, EAA Saleh (2018). Changes in heavy metal accumulation depending on traffic density in some landscape plants. *Pol J Environ Stud* 27:2277–2284

Uka UN, EJD Belford, JN Hogarh (2019). Roadside air pollution in a tropical city: Physiological and biochemical response from trees. *Bull Nat Res Cent* 43:90-101

USEPA (1996). *SW-846 Test Solid Waste, Method 3050B*. Available at: http://www. epa. gov/osw/hazard/testmethods/sw846/pdfs/3050b.pdf

Velikova V, I Yordanov, A Edreva (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci* 151:59–66

Verma V, N Chandra (2014). Biochemical and ultrastructural changes in *Sida cordifolia* L. and *Catharanthus roseus* L. to auto pollution. *Intl Schol Res Notic* 2014; Article 263092

Wang M, H Zhang (2018). Accumulation of heavy metals in roadside soils in urban area and the related impacting factors. *Intl J Environ Res Publ Health* 15:1064–1074

Wekpe VO, GO Chukwu-Okeah, G Kinikanwo (2019). Road construction and trace heavy metals in roadside soils along a major traffic corridor in an expanding metropolis. *Asian Res J Arts Soc Sci* 8:1–10

WHO (1996). *Permissible Limits of Heavy Metals in Soils and Plants*. World Health Organization, Geneva, Switzerland

Zhang F, X Yan, C Zeng, M Zhang, S Shrestha, LP Devkota, T Yao (2012). Influence of traffic activity on heavy metal concentrations of roadside farmland soils in mountainous areas. *Intl J Environ Res Publ Health* 9:1715–1731

Zheng C (2017). Characteristics of heavy metal pollution on roadside soils along highway. *In: AIP Conference Proceedings*, Vol. 1890, pp:1–4. AIP Publishing LLC, Melville, New York, USA