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Status of phytochemicals and antioxidant system of some selective plant species growing in polluted and unpolluted Regions of Makkah Al-Mokorrama, Saudi Arabia

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Key words: Air pollution, Chlorophyll, Phenols, Protein and enzymes

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Abstract

Air pollution is one of the environmental pollutions affects the plant growth and productivity. Over the last decades, the interactions between air pollutant and the plants has been investigated. The present study was carried out to assess the air pollution effects on phytochemicals and antioxidant system in leaves of five plants species including *Aerva javanica, Senna italica, Abutilon pannosum*, *Conocurpus lancifolia* and *Calotropis procerra* growing in polluted and unpolluted areas in Makkah Al-Mokorrama region, Kingdom of Saudi Arabia. Total phenols (TPC), flavonoids concentration (TFC), free radical scavenging capacity (FRSC)(lower DPPH IC_{50} values), protein, proline content, and activities of polyphenoloxidase (PPO), peroxidase (POD) and ascorbate peroxidase (APX) enzymes in leaf were higher in plant species growing in polluted area as compared to unpolluted one. In contrast, photosynthetic pigments such as chlorophyll-a, chlorophyll-b and carotenoids, total soluble sugar (TSS) content, and activity of catalase (CAT) enzyme in leaf were higher in unpolluted site species as compared to polluted one. In polluted area, the highest photosynthetic pigments, TPC, TFC, FRSC, and activity of PPO were observed in *Senna italica*, but the highest proline content and APX activity in *Conocurpus lancifolia*, while the highest TSS content and POD activity in *Calotropis procerra* species, however the highest protein content and CAT activity in *Aerva javanica* and *Abutilon pannosum* species, respectively. These findings suggest that *Senna italica* species has a more effective defense system than the other species studied. The findings of this study will contribute to understand the underlying mechanisms of air pollutants and plants..

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Introduction

Air pollution is defined as the presence of one or more contaminants such as dust, smoke, and varieties of air pollutants which causes concern to human being above all flora and fauna (Lohe *et al.,* 2015). It is mainly caused by human activities such as waste products dumping, fossil fuels incomplete combustion, firewood burning, or other detrimental secondary products that are detrimental to living organisms (Tripathi and Gautam, 2007).

Pollutant discharged from automobiles are expected to have major effects on phenology, flower development, fruiting, leaf senescence and leaf surface wax characteristics, biomass production, seed germination, seedling growth, physiological and biochemical characteristics and plant growth (Narwaria and Kush, 2012; Leghari *et al.,* 2013; Parveen *et al*., 2014). Physiological behavior of plants were greatly affected by the deposition of trace elements, gaseous pollutants, nitrogen oxides (NOx), carbon monoxide (CO), carbon dioxide (CO_2), sulfur dioxide (SO_2) on the leaves (Wittenberghe *et al*., 2013). Air pollutants in different forms are particulate in nature. Air pollutants in gaseous form can penetrate into leaves through the pores of stomata, in which particulates get deposited in plant organs (Patidar *et al.,* 2016).

Over the years, human population, road transportation, vehicular traffic and industries have been increased continuously which has resulted in further increase in the amount of gaseous and particulate pollutants on plants are responsible for altering the ecosystem (Niragau and Davidson, 1986). It is well known that air pollution contribute a great threat to both human health and the environment, and it is estimated that millions of tons of toxic pollutants are released into air each year (Rai, 2013).

Air pollution affects plants through various ways including acidification, eutrophication and ground-level ozone. Chemicals react with air to form compounds that cause harm to vegetation. The evaluation of new antioxidant compounds from polluted medicinal plants has been growing interest in recent times (Radwan *et al.,* 2018). The amounts of secondary metabolites are affected by the biological, physiological, environmental and ecological factors (Ramakrishna and Ravishankar, 2011). Iqbal *et al.* (2015) reported that chlorophyll pigments level in plants species greatly affected due to vehicular activities induced air pollution around road side. Sanaeirad *et al.* (2017) observed that proline and protein and antioxidant enzyme activity were significantly higher in air polluted site than unpolluted one. Makah Al-Mokarama is a highly populated city in KSA in where every year millions of people visited this city due to perform hajj and omrah which causes automobile exhaust emission with high traffic density leads to increase air pollution.

To the best of our knowledge, no research work has been reported on the effects of air pollution on phytochemical and antioxidant system in leaf of *Aerva javanica, Senna italica, Abutilon pannosum*, *Conocurpus lancifolia* and *Calotropis procerra* species growing in polluted and unpolluted areas. Therefore, the study was aimed to analyze the air pollution affected plants species and to check the mechanisms underlying the effects of air pollution on the plants species growing in Makah Al-Mokarama region, KSA.

Materials and methods

Experimental site

The study was conducted on five plant species grown in industrial city (polluted area) of Makkah Al-Mokaroma and the control location (non-polluted) (Ain Shams) about 40 km away from Makkah city center, Saudi Arabia (Fig. 1).

Plant Materials

The fresh leaf samples were collected from the five plant species growing in polluted and unpolluted site (Fig. 2). The studied plants were-

1. *Aerva javanica* (Kapok bush or Dessert cotton)
2. *Senna italica* (Port royal senna or Italian senna)
3. *Abutilon pannosum* (Ragged mallow)

4. *Conocarpus lancifolius* and 5. *Calotropis procerra* (Apple of sodom)

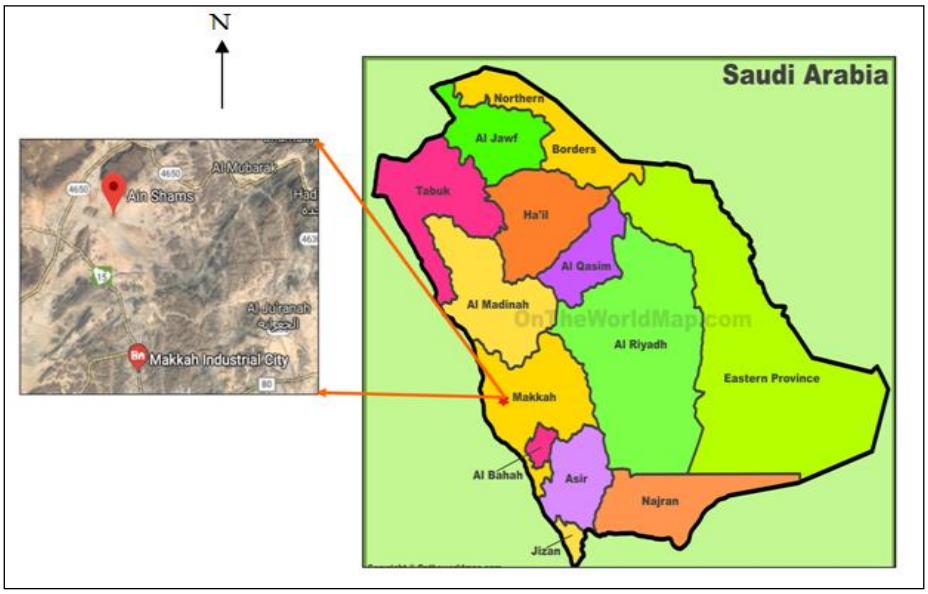

Fig. 1. Location of Makkah, Kingdom of Saudi Arabia, showing of two study sites including (i) Makkah industrial city (polluted) and (ii) Ain Shams (unpolluted).

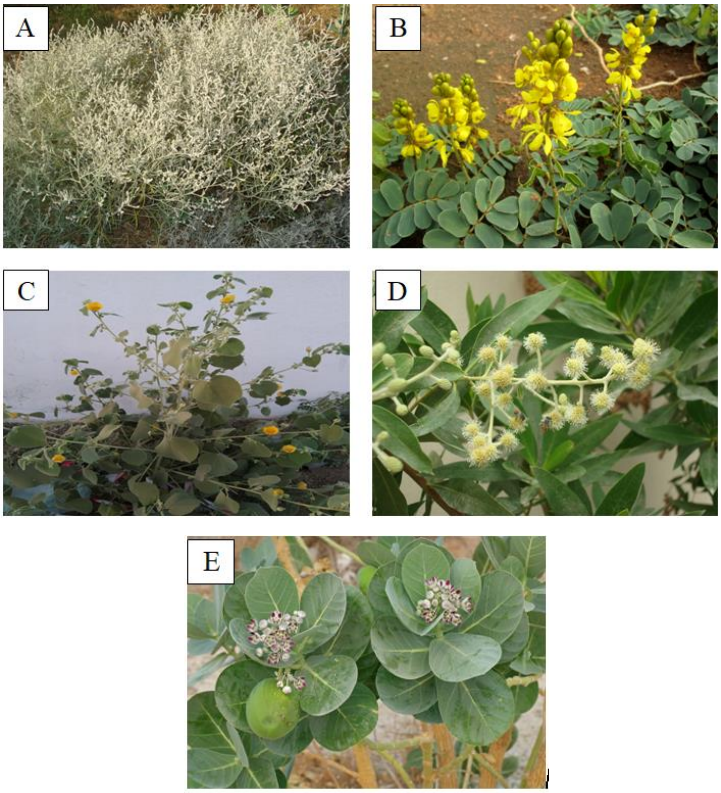

Fig. 2. Plant species showing (A) *Aerva javanica*, (B) *Senna italica*, (C) *Abutilon pannosum*, (D) *Conocarpus lancifolius* and (E) *Calotropis procerra*

Samples collection

Leaf samples were collected from the middle 5th branch of plant from each species with three replicates. Collected leaves were mixed and a random portion was used for chlorophyll, carotenoids, TSS, protein and proline content determinations. While, the other

portion of leaves were kept at –80°C for later enzyme, TPC, TFC and antioxidant activity analysis.

Determination of chlorophyll-a, chlorophyll-b and carotenoids content

Homogenized leaf sample (0.5g) were collected from 3 samples of each plant for measuring chlorophyll-a, chlorophyll-b and carotenoids. Samples were crushed carefully using mortar and pestle. 10 milliliters (ml) of acetone (80%) were added to the sample followed by centrifuging at 5,000×g for 10min. Spectrophotometer (UV-1900) was used to measure the absorbance (UV) of Chlorophyll-a, Chlorophyll-b and carotenoids content at wavelength of 663, 645 and 470nm respectively, according to the method of Lichtenthaler and Wellbum (1983).

Determination of proline content

Free proline content was determined using protocol by Bates *et al.* (1973). Fresh leaf sample (0.5g) was taken and homogenized in 10 ml sulfosalicylic acid (3%) in ice. The homogenate was centrifuged at speed of 11,500×g for 15min. 2ml of filtrate was collected, which was allowed to react with 2 ml acid ninhydrin and 2ml glacial acetic acid.

The mixture was incubated at 100°C for an hr. 4ml toluene was added to the mixture. The absorbance was measured at 520nm. From the standard curve, the amount of proline was determined and expressed as µg/g FW.

Determination of protein content

Protein content in leaf was determined as detailed in Lowry *et al.* (1951) in which bovine serum albumin was used as standard. Protein content in the sample was calculated from the graph by plotting a graph of absorbance vs concentration for standard protein solutions. It was expressed as mg/g FW.

Determination of total soluble sugar

TSS was determined according to the procedure of Dey (1990) with little modification. Randomly taken 0.5g of fresh leaf sample was extracted twice with hot 90% ethanol. The ethanol extracts were then combined. The final volume of the pooled extract was made to 25ml with de-ionized water. A suitable aliquot was taken from the extract and 1ml phenol (5%) and 5ml H_2SO_4 (98%) were added. Final volume of this solution was made to 10ml by adding de-ionized water. Absorbance of final solution was estimated at 485 nm spectrophotometric ally was expressed as µg/g FW.

Preparation of methanol extract of leaf

Leaf sample 0.5g (randomly collected from each replicate) were extracted by shaking at 150 rpm for 12h with 20ml methanol (80%) and filtered with Whatman No. 1. The filtrate designated as methanol extract that will be used for total phenols, total flavonoids and antioxidant activity estimations.

Determination of total phenols concentration

TPC was determined according to Hoff and Singleton (1977). Fifty µl of the methanol extract was mixed with 100µl Folin-Ciocalteu reagent, 850µl of methanol and allowed to stand for 5 min at ambient temperature. A 500 µl of 20% sodium carbonate was added and allowed to react for 30min. Absorbance was measured at 750nm. TPC was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as mg/g FW gallic acid equivalent.

Determination of total flavonoids concentration

TFC was determined by a colorimetric method as described by Zhishen *et al.* (1999). Methanol extract or standard solution (250µl) was mixed with distilled water (1.25ml) and 5% $NaNO_2$ solution (75µl). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150µl), 1 M NaOH (0.5ml) and distilled water (275µl) were added to the mixture 5min later. The absorbance of the solutions at 510nm was then measured. TFC was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catechin and the results expressed as mg/g FW catechin equivalent.

Evaluation of DPPH radical scavenging assay

FRSC of methanol extract of leaf was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method according to Awad *et al.* (2017) with little modification. A methanol extract (0.1ml) was added

to 0.9ml of freshly prepared DPPH methanol solution (0.1mm).

An equal amount of methanol was used as a control. After incubation for 30min at room temperature in the dark, the absorbance (Abs) was measured at 517nm using a spectrophotometer. Activity of scavenging (%) was calculated using the following formula:

DPPH radical scavenging % = [(Abs control – Abs sample)/Abs control] x 100 The inhibition concentration (IC_{50}) was defined as µg phenolics of the test sample that decreases 50% of initial radical. The IC_{50} values were calculated from the dose responses curves.

Enzymes measurements

Crude extract

Two g of leaf sample (randomly collected from each replicate) were homogenized with 20mm Tris–HCl buffer, pH 7.2 (Ali *et al*., 2018). The homogenate was centrifuged at 10,000 rpm for 10min at 4°C. The supernatant (crude extract) was stored at -20°C for peroxidase (POD), polyphenoloxidase (PPO), catalase (CAT) and ascorbate peroxidase (APX) assay.

Peroxidase assay

POD (EC 1.11.1.7) activity was measured as described by Miranda *et al.* (1995). The reaction mixture containing in one ml: 0.008ml of 0.97m H_2O_2, 0.08ml of 0.5m guaiacol, 0.25ml of 0.2m sodium acetate buffer, pH 5.5 and least amount of enzyme preparation. The change in absorbance at 470nm due to guaiacol oxidation was followed for 1min using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which increases the O.D. 1.0 per min under standard assay conditions.

Polyphenoloxidase assay

PPO (EC 1.14.18.1) activity was measured with catechol as a substrate according to the spectrophotometric procedure of Jiang *et al.* (2002). The extract (0.2ml) was rapidly added to 2.8ml of 20mm catechol solution prepared in 0.01m sodium phosphate buffer (pH 6.8). The increase in absorbance at 400nm was recorded for 3min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min.

Catalase assay

CAT activity was determined according to Bergmeyer and Gawehn (1974) 2ml of substrate solution was made up of 25mm H_2O_2 in a 75mm PBS pH 7.0 with 0.5ml crude extract. The absorbance at 240nm was recorded for 1min using a spectrophotometer. 1 unit of enzyme activity was calculated as mentioned above.

Ascorbate peroxidase assay

APX activity was assayed using the method of Nakano and Asada (1987), with some modifications. The mixture contained 50mm potassium phosphate (pH 7.0), 0.25 mM ascorbic acid, 0.05mm EDTA and 0.2mL enzyme extract in a total volume of 2.7mL. After adding 0.3mL of H_2O_2 to a final concentration of 0.2mm, the change in absorbance was monitored at 290 nm. APX activity is expressed as U290, in which U290 = 0.01ΔA 290mg^{-1} protein min^{-1}.

Experimental design

Factorial experiment in a completely randomized design with 3 replications were used, while industry area and plant species were the studied factors.

Statistical analysis

The obtained data of this experiment were statistically analyzed as a completely randomized design with three replicates by analysis of variance (ANOVA) using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by the Duncan's multiple range test at P ≤5%.

Results and discussion

Photosynthetic pigments

Chlorophyll-a content in leaf was significantly higher in the plant species growing in unpolluted area than polluted one, except for *Calotropis procerra* species, in which it was statistically similar (Fig. 3).

Leaf of *Senna italica* plant species retained significantly higher chlorophyll-a content both in polluted and unpolluted areas as compared to other species. In polluted area, there were no significant differences between *Abutilon pannosum* and

Conocurpus lancifolia on Chlorophyll-a content in leaf.

However, in polluted area, there were no significant differences among *Aerva javanica, Abutilon pannosum* and *Calotropis procerra* on Chlorophyll-a content in leaf.

Chlorophyll-b content in leaf was significantly higher in the plant species growing in unpolluted area than polluted one, except for *Senna italica* species, in which it was statistically similar (Fig. 4).

Leaf of *Senna italica* plant species retained significantly higher chlorophyll-a content both in polluted and unpolluted areas compared to other species. In polluted area, there were no significant differences between *Abutilon pannosum, Conocurpus lancifolia* and *Calotropis procerra* on Chlorophyll-b content in leaf. However, in polluted area, there were no significant differences among *Aerva javanica, Abutilon pannosum* and *Calotropis procerra* on Chlorophyll-b content in leaf.

These results are in line with those of Giri *et al.* (2013), Leghari *et al.* (2013), Iqbal *et al.* (2015), Falusi *et al.* (2016) and Pimple (2017), who reported that the photosynthetic pigments such as chlorophyll-a and chlorophyll-b in plant leaves decreased in polluted area as compared to unpolluted one.

The decrease in chlorophyll concentration might be owing to the chlorophyll degradation into phaeophytin by the loss of Mg ions. Also, the deposition of pollutants on the surface of leaves have been observed to cause clogging of stomata which ultimately causes reduction in photosynthetic rate leading to reduction in chlorophyll, and sugar contents (Joshi and Swami, 2009.

Narwaria and Kush, 2012; Prajapati and Tripathi, 2008). Furthermore, pollutants have been reported to inhibit the photosynthetic activity of plants growing in polluted environment resulting in depletion of chlorophyll and carotenoid contents of leaves of plants (Chauhan and Joshi, 2008).

The result of current study is however not in agreement those of Seyyednejad *et al.* (2009) and Assadi *et al.* (2011) who reported an increase of chlorophyll-a and chlorophyll-b in leaf of *Callistemon citrinus* and *Eucalyptus camaldulensis*, respectively under polluted environment than clean one. Carotenoids content in leaf was significantly higher in the plant species growing in unpolluted area than polluted one, except for *Calotropis procerra,* in which it was statistically similar (Fig. 5).

In unpolluted area, *Aerva javanica* and *Senna italia* plant species contained statistically similar but the highest carotenoids content followed by *Abutilon pannosum, Conocurpus lancifolia* and *Calotropis procerra*. However, in polluted area, *Senna italica* plant species retained the highest carotenoids content followed by *Aerva javanica, Abutilon pannosum, Conocurpus lancifolia* and *Calotropis procerra*.

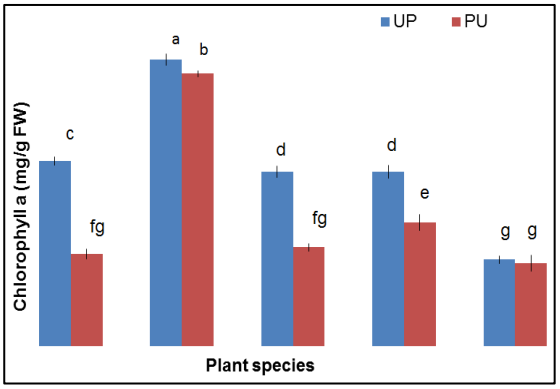

Fig. 3. Comparing chlorophyll a (Chl-a) content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

In this respect, there were no significant differences between *Conocurpus lancifolia* and *Calotropis procerra* on carotenoids content in leaf. The observed decrease in carotenoids in polluted areas are in accordance with those of Ogboru *et al.* (2016) who found that carotenoids concentration was much higher in non-polluted Termilinacatapa region with a

value of 0.36mg/g FW and very lower in polluted Uniben forest reserve with a value of 0.006mg/g FW. Also, Pimple (2017) and Giri *et al.* (2013).

Found that carotenoids content was much higher in unpolluted area as compared to polluted one. However, the result of this study is contradict with those of Seyyednejad *et al.* (2009) and Assadi *et al.* (2011), who observed higher carotenoids content in leaf of *Callistemon citrinus* and *Eucalyptus camaldulensis* species, respectively under polluted condition compared to control.

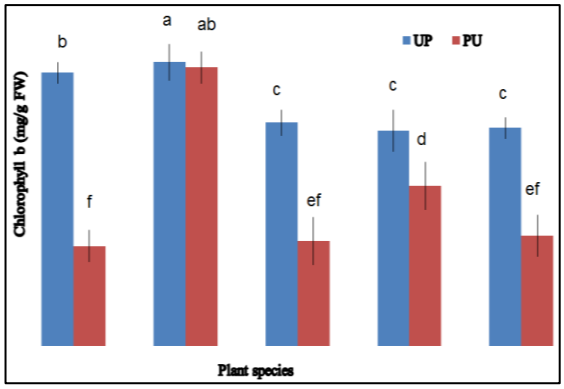

Fig. 4. Comparing chlorophyll-b content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at P ≤ 0.05. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

Antioxidant compounds and antioxidant capacity

TPC content in leaf was significantly higher in the plant species growing in polluted area than unpolluted area plants, except for *Abutilon pannosum,* in which it was statistically similar (Table 1). In unpolluted area, *Senna italica* and *Abutilon pannosum* plant species showed statistically similar but the highest TPC followed by *Aerva javanica, Calotropis procerra* and *Conocurpus lancifolia* plant species. In this respect, there were no significant differences between *Aerva javanica* and *Calotropis procerra* plant species on TPC in leaf. However, in polluted area, *Senna italica* plant species showed the highest TPC in leaf among all plant species.

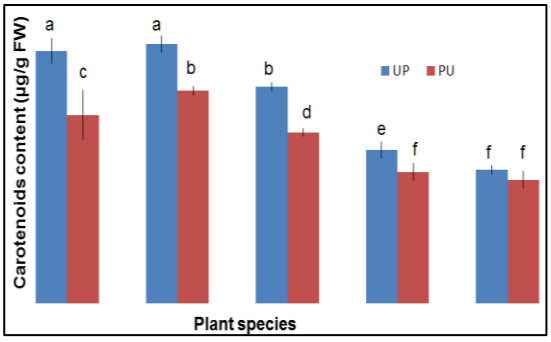

Fig. 5. Comparing carotenoids content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at P ≤ 0.05. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

In this regards, there were no significant differences on TPC in leaf among *Abutilon pannosum, Conocurpus lancifolia* and *Calotropis procerra* plant species. The observed increase in TPC in polluted areas are in accordance with those of Radwan *et al.* (2018) who found that TPC was higher in the plants growing in polluted region and lower in unpolluted site. Similar observation also noted by Marquez-Garcia *et al.* (2012) in *Erica andevalensis* species in Cd induced stress site than control. TFC content in leaf was significantly higher in the plant species growing in polluted area compared to unpolluted one, except for *Abutilon pannosum*, in which it was statistically similar (Table 1). In unpolluted area, *Abutilon pannosum* species showed the highest TFC in leaf followed by *Senna italica, Aerva javanica, Calotropis procerra* and *Conocurpus lancifolia* plant species. In this case, there were no significant differences among *Aerva javanica, Calotropis procerra* and *Conocurpus lancifolia* plant species on TFC. However, in polluted area, *Senna italica* plant species showed the highest carotenoids content in leaf among all plant species. In this regards, there were no

significant differences among *Aerva javanica, Abutilon pannosum, Conocurpus lancifolia* and *Calotropis procerra* plant species on TFC.

These results are in line with those of Radwan *et al.* (2018), who reported that the TFC in plant leaves increased in polluted area than unpolluted one. In addition, Mir *et al.* (2009) found that TPC and TFC in selected plants increased with the increasing of load of pollution across the sites. Similar observation also noted by Marquez-Garcia *et al.* (2012), who observed that TFC in leaf *Erica andevalensis* species was higher in Cd induced stress site than control. The increased of phenolic compound especially flavonoids in polluted area might be due to as a defense element against abiotic stresses including air pollutants (Rezanejad, 2009). Their protective responses may include an increase in antioxidant enzymes and metabolites and induction of protection-related secondary metabolite genes especially flavonoids. FRSC in leaf measured by DPPH method was significantly lower (higher DPPH IC50 value) in the plant species growing unpolluted areas compared to polluted areas growing plants, except for *Senna italica* and *Abutilon pannosum* plant species, in which they were statistically similar (Table 1). In unpolluted area, *Abutilon pannosum* and *Senna italica* plant species showed statistically similar but the highest FRSC (lower DPPH IC50 values) followed by *Aerva javanica, Calotropis procerra* and *Conocurpus lancifolia* plant species. In this case, there were no significant differences among *Aerva javanica* and *Calotropis procerra* plant species on FRSC. However, in polluted area, there were no significant differences among plant species on FRSC in leaf. The observation noted by Marquez-Garcia *et al.* (2012) is an agreement with our results, they found higher antioxidant capacity measured by DPPH method in the leaf of *Erica andevalensis* species in Cd induced stress condition than control. However, the observed higher FRSC in polluted area compared to unpolluted one is in contradiction with those of Keshishian *et al.* (2015) who found that FRSC was higher in unpolluted *Zea mays* plant than the same plant growing in polluted site.

Table 1. Antioxidant compound total phenols concentration (TPC), total flavonoids concentration (TFC) and free radical scavenging capacity (FRSC) (DPPH IC_{50}) in the leaf of five plant species growing in unpolluted and polluted area in Makkah city.

Plant species	Treatment (area)	TPC (mg/g FW)	TFC (mg/g FW)	FRSC
Aerva javanica	Unpolluted	3.12d	0.34cd	16.59b
	Polluted	9.89b	0.75b	5.49c
Senna italica	Unpolluted	6.81c	0.45c	7.67c
	Polluted	11.58a	1.22a	4.73c
Abutilon pannosum	Unpolluted	7.45c	0.67b	6.47c
	Polluted	7.56c	0.82b	5.61c
Conocurpus lancifolia	Unpolluted	1.81e	0.22d	24.35a
	Polluted	6.96c	0.65b	8.86c
Calotropis procerra	Unpolluted	2.94d	0.35cd	17.56b
	Polluted	6.87c	0.81b	9.78c
F-test		***	**	**

Means within each column followed by the same letter are not significantly different at level $P \leq 0.05$.

Protein content

Protein content in leaf was significantly higher in the plant species growing in polluted areas than unpolluted areas plants, except for *Abutilon pannosum*, in which it was statistically similar (Fig. 6). Both in polluted and unpolluted areas, *Aerva javanica* plant species retained the highest protein content followed by *Senna italica, Calotropis procerra, Abutilon pannosum* and *Conocurpus lancifolia* plant species.

The observed increase in protein content in polluted areas are in accordance with those of Sanaeirad *et al.* (2017) who reported that protein content in the plants of polluted area was significantly higher than in the plants those were grown in unpolluted regions. However, our results

contradict with those of Rezanejad (2009) in which the protein content in plants in air polluted areas decreased.

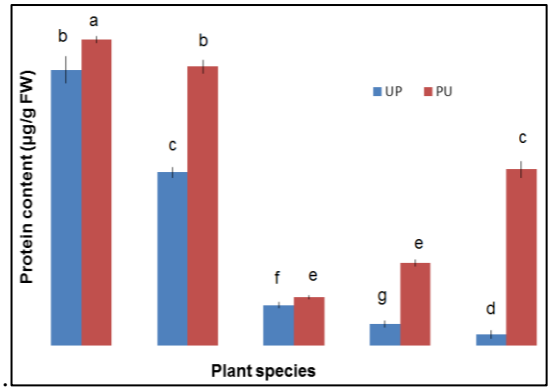

Fig. 6. Comparing protein content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

Proline content

Proline content in leaf was significantly higher in the plant species growing polluted area than unpolluted one, except for *Senna italica,* in which it was statistically similar (Fig. 7). In unpolluted areas, *Abutilon pannosum* plant species retained higher proline content than other plant species. In this case, proline content of *Aerva javanica, Senna italica, Calotropis procerra* and *Conocurpus lancifolia* plant species were statistically similar. However, in polluted area, *Conocurpus lancifolia* plant species contained the highest proline content followed by *Calotropis procerra, Aerva javanica, Abutilon pannosum and Senna italica,* in which *Aerva javanica* and *Abutilon pannosum* species were statistically similar. These results regarding increase of proline content in polluted sites are in agreement with those of Assadi *et al.* (2011) and Patidar *et al.* (2016), who reported that proline content was increased in polluted site *Eucalyptus Camaldulensis* and *Mangofera indica* trees, respectively when compared with unpolluted site trees. Also, Agbaire (2016) reported higher proline content in leaf of some specific plant species under polluted area than unpolluted area, suggesting protective mechanism of plants under stress condition.

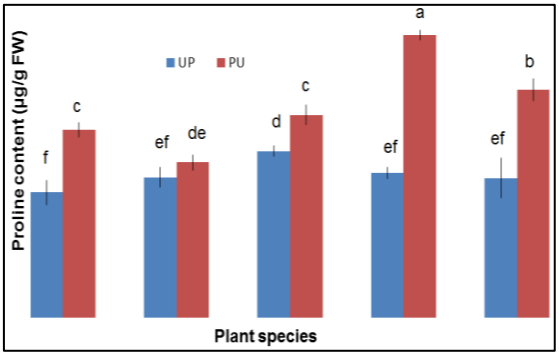

Fig. 7. Comparing proline content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

Total soluble sugar

TSS in leaf was significantly higher in the plant species growing in unpolluted areas compared to the plant species growing in polluted one, except for *Aerva javanica* plant species, in which it was statistically similar in both sites (Fig. 8). In unpolluted area, *Calotropis procerra* plant species retained the highest TSS content followed by *Conocurpus lancifolia, Abutilon pannosum, Senna italica* and *Aerva javanica* species. On the other hand, in polluted area, there were no significant differences among plant species on TSS content. TSS is considered an important constituent and energy source for all living organisms. Plants manufacture such organic substance during photosynthesis and breakdown during respiration (Tripathi and Gautam, 2007). The observed lower in TSS content in leaf in polluted area plants are in accordance with those of Tripathi and Gautam (2007), Seyydenjad and Koochak (2011), Irerhievwie *et al.* (2014) and Agbaire (2016) who reported that TSS content in the plants of polluted area was significantly lower than in the plants those were grown in unpolluted site. The

concentration of TSS is indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution.

Reduction in TSS content in polluted stations can be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration (Fig. 3-5). It has been mentioned that pollutants like SO_2, NO_2 and H_2S under hardening conditions can cause more depletion of soluble sugars in the leaves of plants grown in polluted area. The reaction of sulfite with aldehydes and ketones of carbohydrates can also cause reduction in carbohydrate content (Tripathi and Gautam, 2007). Some researchers showed that TSS content decreased significantly in the sensitive trees to the air pollution. The decrease in TSS content in polluted plant leaf probably corresponded with the photosynthetic inhibition or stimulation of respiration rate (Tzvetkova and Kolarov, 1996). Furthermore, increase of TSS content is a protecting mechanism of leaf it has been shown in Pinto bean in exposure with different concentration of ozone (Dugger and Ting, 1970). The result of current study is however not in agreement those of Seyydnejad *et al.* (2009) and Assadi *et al.* (2011) who reported an accumulation of TSS content in leaf of *Eucalyptus camaldulensis* under polluted environment. It is worthy of note that there would be accumulation or reduction of TSS content.

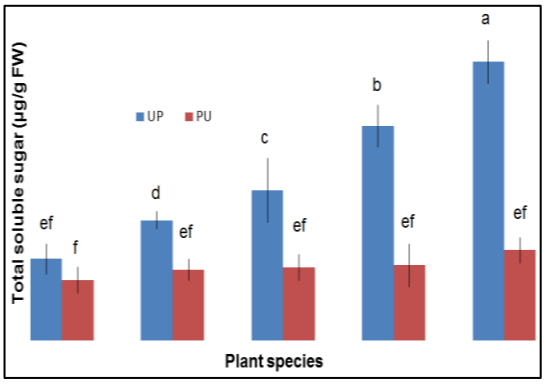

Fig. 8. Comparing total soluble sugar content in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

Antioxidant enzymes activity

Among the all plant species, only *Conocurpus lancifolia* species showed significantly higher PPO activity in leaf in polluted area compared to unpolluted area, in which the other plant species showed relatively stable PPO activity in both sites (Fig. 9). In unpolluted areas, *Conocurpus lancifolia* plant species showed the lowest PPO activity among all the plant species, except for Calotropis *procerra*. In this case, there were no significant differences among *Aerva javanica, Senna italica, Abutilon pannosum* and *Calotropis procerra,* plant species on PPO activity in leaf. However, in polluted area, apparently *Senna italica* plant species showed the highest PPO activity in leaf among all plant species but there were no significant differences among the species.

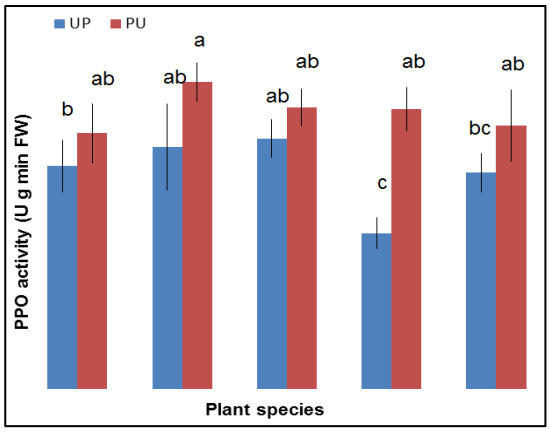

Fig. 9. Comparing polyphenoloxidase (PPO) enzyme activity in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

The higher trend of PPO activity in leaf of species growing in polluted area are in accordance with those of Polovnikova and Voskresenskaya (2008), who reported that this enzyme activity in leaf of red clover (*Trifolium pretense* L.) and meadow-fescue (*Festuca*

pratensis Huds.) species growing in polluted area was higher than the plants growing in unpolluted condition.

Also, Sharma *et al.* (2012) reported the higher PPO activity in leaf of *Raphanus sativus* L. plants under cadmium and mercury metal stress condition. POD activity in leaf was significantly higher in the plant species growing polluted areas than unpolluted areas plants (Fig. 10).

In unpolluted area, POD activity was the highest in the leaf of *Calotropis procerra* plant species followed by *Abutilon pannosum, Aerva javanica, Conocurpus lancifolia* and *Senna italia* plant species. In this regards, there were no significant differences on POD activity between *Abutilon pannosum* and *Aerva javanica* or *Conocurpus lancifolia* and *Senna italica* species. While in polluted area, *Calotropis procerra* plant species showed the highest POD activity followed by *Abutilon pannosum, Aerva javanica, Conocurpus lancifolia* and *Senna italica* plant species. In this regards, there were no significant differences on POD activity among *Aerva javanica, Conocurpus lancifolia* and *Senna italica* species.

These results are in line with those of Polovnikova and Voskresenskaya (2008), who reported that POD activity in leaf of *Trifolium pretense* L. and *Festuca pratensis* Huds. species growing in polluted area was higher compared to unpolluted one. However, our results contradict with those of Shyam *et al.* (2008) in which the POD activity in plant leaf decreased in industrial area compared to residential area. APX activity in leaf was significantly higher in the plant species growing in polluted area than unpolluted one (Fig. 11). In unpolluted areas, *Abutilon pannosum* plant species showed the highest APX activity followed by *Calotropis procerra, Senna italica, Conocurpus lancifolia* and *Aerva javanica,* plant species, in which there were no significant differences among *Calotropis procerra, Senna italica* and *Conocurpus lancifolia* species. While in polluted area, *Conocurpus lancifolia* plant species showed the highest APX activity followed by *Calotropis procerra, Senna italica, Abutilon pannosum* and *Aerva javanica* species.

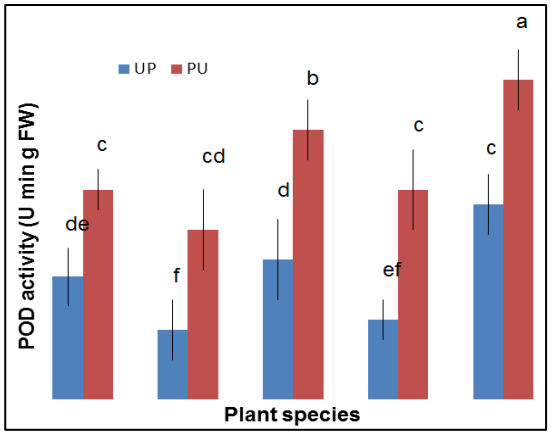

Fig. 10. Comparing peroxidase (POD) enzyme activity in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

These results regarding increase of APX activity in stress condition or polluted sites are in partial agreement with those of Zaimoglu *et al.* (2011), who reported that APX activity was higher in polluted site plants as compared non-contaminated site. CAT activity in leaf of *Conocurpus lancifolia* and *Calotropis procerra* were significantly higher in the plant species growing polluted areas than unpolluted areas plants (Fig. 12). While CAT activity was relatively stable in the leaf of *Aerva javanica, Senna italica* and *A butilon pannosum* plant species both in polluted and unpolluted areas. In unpolluted areas, *Abutilon pannosum* and *Conocurpus lancifolia* plant species showed statistically similar but the highest CAT activity in leaf followed by *Calotropis procerra, Senna italica* and *Aerva javanica* plant species, in which there were no significant differences between *Senna italica* and *Aerva javanica* species. While in polluted area, *Abutilon pannosum* plant species showed the highest CAT activity followed by *Conocurpus lancifolia*, *Calotropis procerra, Senna*

italica and *Aerva javanica* plant species. In this case, there were no significant differences among *Calotropis procerra, Senna italica* and *Aerva javanica* plant species on CAT activity in leaf.

This is close conformity with findings of Polovnikova and Voskresenskaya (2008) and Zaimoglu *et al.* (2011), who reported that CAT activity was higher in non-stress condition in all plant species compared to stress site.

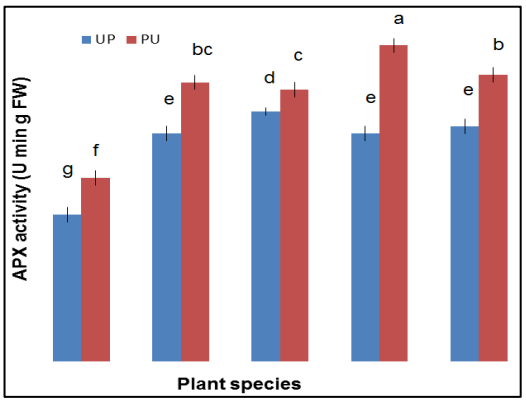

Fig. 11. Comparing ascorbate peroxidase (APX) enzyme activity in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at P ≤ 0.05. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

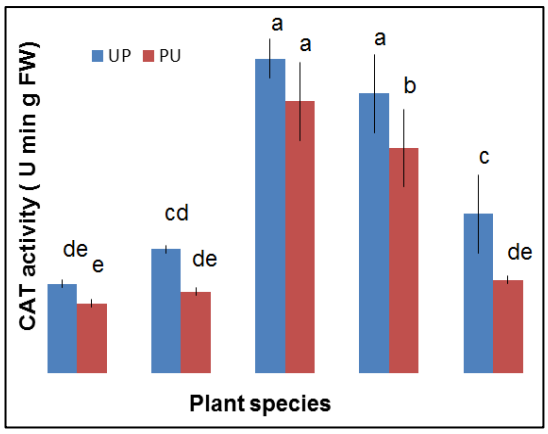

Fig. 12. Comparing catalase (CAT) enzyme activity in the leaf of five plant species growing in polluted (PU) and unpolluted (UP) areas in Makkah city. Vertical bar shows standard deviations (n=3) and values followed by different letters are significantly different according to Duncan's multiple range test at P ≤ 0.05. AJ, SI, AP, CL, CP, UP and PU refereeing to *Aerva javanica, Senna italica, Abutilon pannosum, Conocurpus lancifolia, Calotropis procerra,* unpolluted and polluted respectively.

Conclusion

The present study showed that phytochemical of some specific plants was greatly varied due to air pollution in polluted aria as compared to unpolluted site. Antioxidant network (except for CAT activity), protein and proline content were found higher in leaf in most of the species growing in polluted area compared to unpolluted one. However, photosynthetic pigments and TSS content in leaf were observed lower in polluted site species as compared to unpolluted one. These findings suggest that *Senna italica* species has a more effective defense system than the other species studied. The findings of this study will contribute to understand the underlying mechanisms of air pollutants and plants.

References

Agbaire PO. 2016. Impact of air pollution on proline and soluble sugar content of selected plant species. Chemistry and Materials Research **8(5),** 72-76.

Ali MA, Awad MA, Al-Qurashi AD, El-Shishtawy RM, Mohamed SA. 2019. Quality and biochemical changes of 'Grand Nain' bananas during shelf life, J. K. A. U. Met. Environ. Arid Land Agric. Sci. **28(1),** 41-56.

Ao C, Li A, Elzaawely AA, Xuan TD, Tawata S. 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. Food Control 19, 40-948.

Assadi A, Pirbalouti AG, Malekpoor F, Teimori N, Assadi L. 2011. Impact of air pollution on physiological and morphological characteristics of *Eucalyptus camaldulensis* Den. Journal of Food, Agriculture & Environment **9(2),** 676-679.

Awad MA, Alqurashi AD, Mohamed SA, El-Shishtawy RM, Ali MA. 2017. Postharvest chitosan, gallic acid and chitosan gallate treatments

effects on shelf life quality, antioxidant compounds, free radical scavenging capacity and enzymes activities of 'Sukkari' bananas. Journal of Food Science and Technology **54,** 447-457.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of proline for water stress studies. Plant Soil **39,** 205-207.

Bergmeyer HU, Gawehn K. 1974. Methods of enzymatic analysis. Verlag Chemistry **4,** 23-32.

Chauhan A, Joshi PC. 2008. Effect of ambient air pollution on photosynthetic pigments on some selected trees in urban area. Ecology Environment and Conservations **14,** 23-27.

Dey PM. 1990. Oligosaccharides. In: Dey PM, Harborne JB. (eds.): Methods in Plant Biochemistry, vol. 2, Carbohydrates. Academic Press, London 189-218.

Dugger WM, Ting IP. 1970. Air pollution oxidant-their effects on metabolic processes in plants. Annual Review on Plant Physiology **21,** 215-234.

Falusi BA, Odedokun OA, Abubakar A, Agoh A. 2016. Effects of dumpsites air pollution on the ascorbic acid and chlorophyll contents of medicinal plants. Cogent Environmental Science **2(1170585),** 1-13.

Giri S, Shrivastava D, Deshmukh K, Dubey P. 2013. Effect of Air Pollution on Chlorophyll Content of Leaves. Current Agricultural Research **1(2),** 93-98.

Hoff JF, Singleton KI. 1977. A method for determination of tannin in foods by means of immobilized enzymes. Journal of Food Science **42,** 1566-1569.

Iqbal MZ, Shafig M, Zaidi SQ, Athar M. 2015. Effect of automobile pollution on chlorophyll content of roadside urban trees. Global Journal of Environmental Science and Management **1(4),** 283-296.

Irerhievwie GO, Akpoghelie JO, Esiefarienrhe E. 2014. Evaluation of some plant species for soluble sugar and air pollution tolerance index in Oleh Metropolis, Isoko South L. G. A., Delta State, Nigeria. Journal of Emerging Trends in Engineering and Applied Science **5(5),** 323-328.

Jiang YM, Zhang ZQ, Joyce DC, Ketsa S. 2002. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biology and Technology **26,** 241-252.

Joshi PC, Swami A. 2009. Air pollution induced changes in the photosynthetic pigments of selected plant species. Journal of Environment and Biology **30,** 295-298.

Keshishian N, Amjad L, Yahyaabadi S. 2015. Comparative study of phytochemical parameters and antioxidant activity of accumulated *Zea mays* from different regions of Isfahan. Journal of Biodiversity and Environmental Sciences **6(5),** 367-373.

Leghari SK, Zaid MA, Sarangzai AM, Faheem M, Shawani GR, Ali W. 2013. Effect of road side dust pollution on the growth and total chlorophyll contents in *Vitis vinifera* L (grape). African Journal of Biotechnology **13(11),** 1237-1242.

Lichtenthaler HK, Wellburn AR. 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions **11,** 591-603.

Lohe RN, Tyagi B, Singh V, Tyagi PK, Khanna DR, Bhutiani R. 2015. A comparative study for pollution tolerance index of some terrestrial plant species. Global Journal of Environmental Science and Management **1,** 315-324.

Lowry OH, Rosebrough NJ, Farr AL, Randall RL. 1951. Protein measurement with Folin phenol reagent. Journal of Biological Chemistry **193,** 265-275.

Marquez-Garcia B, Fernandez-Recamales MA, Cordoba F. 2012. Effects of cadmium on phenolic composition and antioxidant activities of *Erica andevalensis*. Journal of Botany **936950,** 1-6.

Mir AQ, Yazdani T, Ahmad S, Yunus M. 2009. Total Flavonoids and Phenolics in *Catharanthus roseus* L. and *Ocimum sanctum* L. as Biomarkers of Urban Auto Pollution. Caspian Journal of Environmental Science **7(1),** 9-16.

Miranda MV, Lahor HMF, Cascone O. 1995. Horseradish peroxidase extraction and purification by aqueous two-phase partition. Applied Biochemistry and Biotechnology **53,** 147-154.

Nakano Y, Asada K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiology **28,** 131-140.

Narwaria YS, Kush K. 2012. Environmental assessment of air pollution on roadside plants species at Dehradun, Uttrakhand, India. Journal of Environmental Research and Development **7,** 710-714.

Niragau JO, Davidson CL. 1986. Toxic Metals in the Atmosphere. Jon Wiley and Sons, New York.

Ogboru RO, Okolie LP, Idibie CA. 2016. Impact of air pollution on carotenoid and chlorophyll contents in three forest reserves in Edo State, Nigeria. International Journal of Scientific Research **5(1),** 616-622.

Parveen S, Iqbal MZ, Shafiq M, Athar M. 2014. Effect of automobile polluted soil on early seedling growth performance of Neem (*Azadirachta indica* A. Juss.). Advanced Environmental Research **3,** 1-9.

Patidar S, Bafna A, Batham AR, Panwar K. 2016. Impact of urban air pollution on photosynthetic pigment and proline content of plants growing along the A. Broad Indore City, India. International Journal of Current Microbiology and Applied Sciences **5(3),** 107-113.

Pimple NS. 2017. Adverse effect of air pollutants on the chlorophyll content in leaves from Pune, Maharashtra (India). International Journal of Pharmaceutical Sciences Review and Research **44(2),** 131-135.

Polovnikova MG, Voskresenskaya OL. 2008. Activities of antioxidant system components and polyphenol oxidase in ontogeny of lawn grasses under Megapolis conditions. Russian Journal of Plant Physiology **55(5),** 699-705.

Prajapati SK, Tripathi BD. 2008. Seasonal variation of leaf dust accumulation and pigment content in plant species exposed to urban particulates pollution. Journal of Environmental Quality **37,** 865-870.

Radwan AM, Reyad NF, Donia ARM, Ganaie MA. 2018. Comparative studies on the effect of environmental pollution on secondary metabolite contents and genotoxicity of two plants in Asir area, Saudi Arabia. Tropical Journal of Pharmaceutical Research **17(8),** 1599-1605.

Rai PK. 2013. Environmental magnetic studies of particulates with special reference to biomagnetic monitoring using roadside plant leaves. Atmospheric Environment **72,** 113-129.

Ramakrishna A, Ravishankar GA. 2011. Influence of Abiotic Stress Signals on Secondary Metabolites in Plants. Plant Signaling and Behavior **6,** 1720-1731.

Rezanejad F. 2009. Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). Grana **48,** 205-0213.

Sanaeirad H, Majd A, Abbaspour H, Peyvandi M. 2017. The effect of air pollution on proline and protein content and activity of nitrate reductase enzyme in *Laurus nobilis* L. plants. Journal of Molecular Biology Research **7(1),** 99-105.

Seyyednejad SM, Koochak H. 2011. A Study on Air pollution effect on *Eucalyptus camoldulensis*. International Conference on Environmental, Biomedical and Biotechnology **16,** 98-101.

Seyyednejad SM, Niknejad M, Yusefi M. 2009. The effect of air pollution on some morphological and biochemical factors of *Callistemon citrnus* in Petrochemical Zone in South Iran. Asian Journal of Plant Science **8(8),** 562-565.

Sharma N, Hundal GS, Sharma I, Bhardwaj R. 2012. Effect of 24-epibrassinolide on protein content and activities of glutathione-s-transferase and polyphenol oxidase in *Raphanus sativus* L. plants

under cadmium and mercury metal stress. Terrestrial and Aquatic Environmental Toxicology **6(1),** 1-7.

Tripathi AK, Gautam M. 2007. Biochemical parameters of plants as indicators of air pollution. Journal of Environmental Biology **28,** 127-132.

Tzvetkova N. Kolarov D. 1996. Effect of air pollution on carbohydrate and nutrient concentrations in some deciduous tree species. Bulgarian Journal of Plant Physiology **22,** 53-63.

Wittenberghe SV, Alonso L, Verrelst J, Hermans I, Delegido J, Veroustraete F, Valcke R, Moreno J, Samson R. 2013. Upward and downward solar-induced chlorophyll fluorescence yield indices of four tree species as indicators of traffic pollution in Valencia. Environmental Pollution **173,** 29-37.

Zaimoglu Z, Koksal N, Basci N, Kesici M, Gulen H, Budak F. 2011. Antioxidative enzyme activities in *Brassica juncea* L. and *Brassica oleracea* L. plants under chromium stress. Journal of Food, Agriculture and Environment **9(1),** 676-679.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry **64,** 555-559.